# The Flavor and Fragrance High Production Volume Consortia

## The Terpene Consortium



## Robust Summaries for Estragole

**Estragole** **CAS No. 140-67-0**

## FFHPVC Terpene Consortium Registration Number

**Submitted to the EPA under the HPV Challenge Program by:**
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**The evaluation of the quality of the following data uses a systematic approach described by Klimisch [Klimisch *et al*., 1996]. Based on criteria relating to international testing standards for categorizing data reliability, four reliability categories have been established. The following categories are:**

- Reliability code 1.Reliable without restrictions
- Reliability code 2.Reliable with restrictions
- Reliability code 3.Not reliable
- Reliability code 4.Not assignable

# 1 CHEMICAL AND PHYSICAL PROPERTIES

## 1.1 Melting Point

| | |
|---|---|
| **Substance Name** | Estragole |
| **CAS No.** | 140-67-0 |
| **Method/guideline** | Comparison of experimental melting points for a group of structurally related allylalkoxybenzene and propenylalkoxybenzene dervatives |
| **GLP** | No |
| **Melting Point** | 1 °C |
| **Comment on method** | The incremental change in experimental melting point between 4-methoxy- (21 °C) and 3,4 dimethoxy-1-propenylbenzene (16-17 °C) is 4-5 °C. Based on these data and the fact that 3,4-dimethoxy-2-propenylbenzene exhibits a melting point of -4°C |

| | |
|---|---|
| | (Chemical Rubber Handbook), the melting point of 4-methoxy-2-propenylbenzene (estragole) is estimated to be 1 °C |
| **Data Qualities Reliabilities** | Reliability code 2. Iteration of melting point data based on known changes in chemical structure is reliable provided data is available for a sufficient number of congeners.. |
| **References** | Chemical Rubber handbook, 2004. |

| | |
|---|---|
| **Substance Name** | Estragole |
| **CAS No.** | 140-67-0 |
| **Method/guideline** | Calculated/Mean or weighted (adapted Stein and Brown method) |
| **GLP** | No |
| **Melting Point** | -1.19 °C |
| **Data Qualities Reliabilities** | Reliability code 4. Not assignable. |
| **Remarks for Data Reliability** | Code 4. Calculated. |
| **References** | MPBPVP EPI Suite (2000) U S Environmental Protection Agency. |

## 1.2 Boiling Point

| | |
|---|---|
| **Substance Name** | Estragole |
| **CAS No.** | 140-67-0 |
| **GLP** | Ambiguous |
| **Boiling Point** | 216 deg C |
| **Pressure** | 764 |
| **Pressure Unit** | mm Hg |
| **Data Qualities Reliabilities** | Reliability code 2. Reliable with restriction. |
| **Remarks for Data Reliability** | Code 2. Acceptable, well-documented publication/study report, which meets basic scientific principles. |
| **References** | Merck Index (1998) The Merck Index, 12th edition, Merck & Co., Inc. Whitehouse Station, NJ. |

| | |
|---|---|
| **Substance Name** | Estragole |
| **CAS No.** | 140-67-0 |
| **GLP** | Ambiguous |

| | |
|---|---|
| **Boiling Point** | 216 °C |
| **Pressure** | 760 |
| **Pressure Unit** | mm Hg |
| **Data Qualities Reliabilities** | Reliability code 2. Reliable with restriction. |
| **Remarks for Data Reliability** | Code 2. Acceptable, well-documented publication/study report, which meets basic scientific principles. |
| **References** | Fragrance Materials Association (FMA) Reported values for boiling point of estragole. |

| | |
|---|---|
| **Substance Name** | Estragole |
| **CAS No.** | 140-67-0 |
| **Method/guideline** | Adapted Stein and Brown method |
| **GLP** | No |
| **Boiling Point** | 209.93 °C |
| **Data Qualities Reliabilities** | Reliability code 4. Not assignable. |
| **Remarks for Data Reliability** | Code 4. Calculated. |
| **References** | MPBPVP EPI Suite (2000) U S Environmental Protection Agency. |

## 1.3 Vapor Pressure

| | |
|---|---|
| **Substance Name** | Estragole |
| **CAS No.** | 140-67-0 |
| **Method/guideline** | Experimental |
| **GLP** | No |
| **Year** | 1947 |
| **Vapor Pressure** | 1 mm Hg |
| **Temperature** | 52.6 °C |
| **Data Qualities Reliabilities** | Reliability code 2. Reliable with restriction. |
| **Remarks for Data Reliability** | Code 2. Acceptable, well-documented publication/study report, which meets basic scientific principles. |

| | |
|---|---|
| **References** | Stull D.R. (1947) Vapor pressure of pure substances. Organic Compounds. Ind Eng Chem., 39, 517-540. |

| | |
|---|---|
| **Substance Name** | Anethole (isomer unspecified –surrogate for estragole) |
| **CAS No.** | 104-46-1 |
| **Remarks for Substance** | Data is for anethole, isomer unspecified |
| **Method/guideline** | Measured |
| **GLP** | Ambiguous |
| **Vapor Pressure** | 0.041 mm Hg (5.45 Pa) |
| **Temperature** | 21 °C (294 K) |
| **Data Qualities Reliabilities** | Reliability code 2. Reliable with restriction. |
| **Remarks for Data Reliability** | Code 2. Basic data given: comparable to guidelines/standards. |
| **References** | Daubert T.E. and Danner, R.P. (1989) Physical and Thermodynamic Properties of Pure Chemicals Data Compilation. Taylor and Francis, Washington, DC.418 |

| | |
|---|---|
| **Substance Name** | Estragole |
| **CAS No.** | 140-67-0 |
| **Method/guideline** | Calculated |
| **Vapor Pressure** | 0.09 mm Hg (12 Pa) |
| **Temperature** | 20 °C |
| **Data Qualities Reliabilities** | Reliability code 4. Not assignable. |
| **Remarks for Data Reliability** | Code 4. Calculated. |
| **References** | Fragrance Materials Association (FMA) Reported values of vapor pressure for estragole. Unpublished report. |

| | |
|---|---|
| **Substance Name** | Anethole (isomer unspecified –surrogate for estragole) |
| **CAS No.** | 104-46-1 |
| **Remarks for Substance** | Data is for *trans*-anethole |
| **Method/guideline** | Calculated |
| **Vapor Pressure** | 0.05 mm Hg (6.67 Pa) |
| **Temperature** | 20 °C |

| | |
|---|---|
| **Data Qualities Reliabilities** | Reliability code 4. Not assignable. |
| **Remarks for Data Reliability** | Code 4. Calculated. |
| **References** | Fragrance Materials Association (FMA) Reported values of vapor pressure for trans-anethole. Unpublished report. |

## 1.4 n-Octanol/Water Partition Coefficients

| | |
|---|---|
| **Substance Name** | Estragole |
| **CAS No.** | 140-67-0 |
| **Method/guideline** | Calculated |
| **Log Pow** | 3.47 |
| **Data Qualities Reliabilities** | Reliability code 4. Not assignable. |
| **Remarks for Data Reliability** | Code 4. Calculated. |
| **References** | KOWWIN EPI Suite (2000) U.S. Environmental Protection Agency. |

## 1.5 Water Solubility

| | |
|---|---|
| **Substance Name** | Estragole |
| **CAS No.** | 140-67-0 |
| **Method/Guideline** | Measured |
| **GLP** | Ambiguous |
| **Year** | 1992 |
| **Value (mg/L) at Temperature** | 178 mg/L at 25 °C |
| **Data Qualities Reliabilities** | Reliability code 2. Reliable with restriction. |

| | |
|---|---|
| **Remarks for Data Reliability** | Code 2. Basic data given: comparable to guidelines/standards. |
| **References** | WSKOWIN EPI Suite (2000a) U S Environmental Protection Agency (Yalkowski, S.H. and Dannenfelser, R.M., 1992) |

| | |
|---|---|
| **Substance Name** | Anethole (isomer unspecified –surrogate for estragole) |
| **CAS No.** | 104-46-1 |
| **Remarks for Substance** | Data is for anethole, isomer unspecified |
| **Method/Guideline** | Measured |
| **GLP** | No |
| **Value (mg/L) at Temperature** | 111 mg/L at 25 °C |
| **Data Qualities Reliabilities** | Reliability code 2. Reliable with restriction. |
| **Remarks for Data Reliability** | Code 2. Basic data given: peer reviewed reference |
| **References** | WSKOW EPI Suite (2000a) U S Environmental Protection Agency (Yalkowski S.H., and Dannenfelser, R.M., 1992) |

| | |
|---|---|
| **Substance Name** | Estragole |
| **CAS No.** | 140-67-0 |
| **Method/Guideline** | Calculated |
| **Remarks for Test Conditions** | Used an estimated log Kow of 3.47 |
| **Value (mg/L) at Temperature** | 84.55 mg/L at 25 °C |
| **Data Qualities Reliabilities** | Reliability code 4. Not assignable. |
| **Remarks for Data Reliability** | Code 4. Calculated. |
| **References** | WSKOWIN EPI Suite (2000b) US Environmental Protection Agency. |

# 2 ENVIRONMENTAL FATE AND PATHWAYS

## 2.1 Photodegradation

| | |
|---|---|
| **Substance Name** | Estragole |
| **CAS No.** | 140-67-0 |
| **Method/guideline** | Calculated |
| **Test Type** | AOPWIN |
| **Halflife t1/2** | 2.36 hours |
| **Data Qualities Reliabilities** | Reliability code 4. Not assignable. |
| **Remarks for Data Reliability** | Code 4. Calculated. |
| **References** | AOPWIN EPI Suite (2000) US Environmental Protection Agency. |

## 2.2 Biodegradation

| | |
|---|---|
| **Substance Name** | Estragole |
| **CAS No.** | 140-67-0 |
| **Remarks for Substance** | Data is for 3-methoxy-4-hydroxyallylbenzene |
| **Method** | OECD Guideline 301B |
| **Test Type** | Sealed vessel test ($CO_2$ production test) |
| **Year** | 1994 |
| **Innoculum** | 10% by volume of secondary effluent from an unacclimatized activated sludge |
| **Remarks for Test Conditions** | The test concentration was nominal 10 mg/L organic carbon with a test temperature range of 17-22 °C. The mean percentage biodegradation was calculated from 4 vessels on day 28. |
| **Degradation % After Time** | 100.4% (98.0-102.8-%) |
| **10 day window criteria** | Yes |
| **Total degradation** | Yes |

| | |
|---|---|
| **Conclusion Remarks** | Substances is classified as readily and ultimately biodegradable. |
| **Data Qualities Reliabilities** | Reliability code 1. Reliable without restriction. |
| **Remarks for Data Reliability** | Code 1. Guideline study. |
| **Reference** | Quest International, Inc. (1994a) The ultimate and readily biodegradation of eugenol. Unpublished report. |

| | |
|---|---|
| **Substance Name** | Anethole (isomer unspecified –surrogate for estragole) |
| **CAS No.** | 104-46-1 |
| **Remarks for Substance** | Data is for *p*-(2-propenyl)anisole isomer, anethole |
| **Method** | OECD Guideline 301B |
| **Test Type** | Sealed vessel test (CO2 production test) |
| **Year** | 1994 |
| **Innoculum** | 10% by volume of secondary effluent from an unacclimatized activated sludge |
| **Remarks for Test Conditions** | The test concentration was nominal 10 mg/L organic carbon with a test temperature range of 20-24 °C. The mean percentage biodegradation was calculated from 4 vessels on day 28. |
| **Degradation % After Time** | 91.0% (90.7-91.2%) |
| **10 day window criteria** | Yes |
| **Total degradation** | Yes |
| **Conclusion Remarks** | Anethole is classified as readily and ultimately biodegradable. |
| **Data Qualities Reliabilities** | Reliability code 1. Reliable without restriction. |
| **Remarks for Data Reliability** | Code 1. Guideline study. |
| **Reference** | Quest International, Inc. (1994b) The ultimate and readily biodegradation of anethole. Unpublished report. |

| | |
|---|---|
| **Substance Name** | Estragole |
| **CAS No.** | 140-67-0 |
| **Method** | Calculated |
| **Test Type** | BIOWIN |
| **Results** | Probability of rapid biodegradation - linear model 0.8636 - nonlinear 0.9766. Expert survey results - Ultimate survey model: 2.7387 (weeks-months); Primary survey model: 3.6425 (days-weeks) |

| | |
|---|---|
| **Data Qualities Reliabilities** | Reliability code 4. Not assignable. |
| **Remarks for Data Reliability** | Code 4. Calculated. |
| **Reference** | BIOWIN EPI Suite (2000) U S Environmental Protection Agency (Meylan W., 1994). |

## Fugacity

| | |
|---|---|
| **Substance** | Estragole |
| **CAS** | 140-67-0 |
| **Model Conditions** | 25 C, 1000 lbs. |
| **Test Type** | Environmental Equilibrium Partitioning Model |
| **Method** | Mackay |
| **Model Used (title, version, date)** | EQC Fugacity Level III |
| **Input parameters** | MW (148.21), VP(0.041 mm Hg), log Kow (3.47), water solubility (111 mg/L), MP (1 °C), BP (216°C) |
| **Year** | 2000 |
| **Media** | Air-Water-Soil-Sediment Partition Coefficients |
| **Model data and results** | Compartment half-lives, hours: Air=3.92; Water=900;Soil=900;Sediment=3600 |
| **Estimated Distribution and Media Concentration** | Air=0.391%<br>Water=25.1%<br>Soil=73.4%<br>Sediment=1.12% |
| **Conclusion remarks** | Substance is predicted to persist in the environment for 574 hours. |
| **Reliabilities** | Reliability code 4. Not assignable. |
| **Remarks for Data Reliability** | The data are obtained by a recognized fugacity calculation method. Data are considered<br>reliable with restriction because this method does not allow for biodegradation or<br>metabolism. |
| **References** | Mackay D., A.DiGuardo, S.Paterson and C.E.Cowan (1996b) Evaluating the fate of a variety of types of chemicals using the EQC model. Environmental Toxicology and Chemistry, 15(9), 1627-1637. |

| | |
|---|---|
| **Substance Name** | Estragole |

| | |
|---|---|
| **CAS No.** | 140-67-0 |
| **Model Conditions** | 25 °C, 100,000 pounds |
| **Test Type** | Environmental Equilibrium Partitioning Model |
| **Method** | Mackay |
| **Model Used** | Level III |
| **Input Parameters** | MW, calc. log Kow, exp.water solubility, calculated MP, exp. BP & VP |
| **Media** | Air |
| **Estimated Distribution and Media Concentration** | 0.556% |
| **Remarks** | Half-life = 3.92 hours |
| **Data Qualities Reliabilities** | Reliability code 2. Reliable with restriction. |
| **Remarks for Data Reliability** | The data are obtained by a recognized fugacity calculation method. Data are considered reliable with restriction because this method does not allow for biodegradation or metabolism. |
| **References** | Mackay D., A.DiGuardo, S.Paterson, G.Kicsi and C.E.Cowan (1996a, 1996b) Assessing the fate of new and existing chemicals: a five-stage process & Evaluating the fate of a variety of types of chemicals using the EQC model. Env. Tox.& Chem., 15(9), 1618-1637. |

| | |
|---|---|
| **Substance Name** | Estragole |
| **CAS No.** | 140-67-0 |
| **Model Conditions** | 25 °C, 100,000 pounds |
| **Test Type** | Environmental Equilibrium Partitioning Model |
| **Method** | Mackay |
| **Model Used** | Level III |
| **Input Parameters** | MW, log Kow, water solubility, calculated MP & VP |
| **Media** | Water |
| **Estimated Distribution and Media Concentration** | 19.7% |
| **Remarks** | Half-life = 900 hours |
| **Data Qualities Reliabilities** | Reliability code 2. Reliable with restriction. |
| **Remarks for Data Reliability** | The data are obtained by a recognized fugacity calculation method. Data are considered reliable with restriction because this method does not allow for biodegradation or metabolism. |

| | |
|---|---|
| **References** | Mackay D., A.DiGuardo, S.Paterson, G.Kicsi and C.E.Cowan (1996a, 1996b) Assessing the fate of new and existing chemicals: a five-stage process & Evaluating the fate of a variety of types of chemicals using the EQC model. Env. Tox.& Chem., 15(9), 1618-1637. |

| | |
|---|---|
| **Substance Name** | Estragole |
| **CAS No.** | 140-67-0 |
| **Model Conditions** | 25 °C, 100,000 pounds |
| **Test Type** | Environmental Equilibrium Partitioning Model |
| **Method** | Mackay |
| **Model Used** | Level III |
| **Input Parameters** | MW, log Kow, water solubility, calculated MP & VP |
| **Media** | Soil |
| **Estimated Distribution and Media Concentration** | 78.8% |
| **Remarks** | Half-life = 900 hours |
| **Data Qualities Reliabilities** | Reliability code 2. Reliable with restriction. |
| **Remarks for Data Reliability** | The data are obtained by a recognized fugacity calculation method. Data are considered reliable with restriction because this method does not allow for biodegradation or metabolism. |
| **References** | Mackay D., A.DiGuardo, S.Paterson, G.Kicsi and C.E.Cowan (1996a, 1996b) Assessing the fate of new and existing chemicals: a five-stage process & Evaluating the fate of a variety of types of chemicals using the EQC model. Env. Tox.& Chem., 15(9), 1618-1637. |

| | |
|---|---|
| **Substance Name** | Estragole |
| **CAS No.** | 140-67-0 |
| **Model Conditions** | 25 °C, 100,000 pounds |
| **Test Type** | Environmental Equilibrium Partitioning Model |
| **Method** | Mackay |
| **Model Used** | Level III |
| **Input Parameters** | MW, log Kow, water solubility, calculated MP & VP |
| **Media** | Sediment |
| **Estimated Distribution and Media Concentration** | 0.88% |

| | |
|---|---|
| **Remarks** | Half-life = 3600 hours |
| **Data Qualities Reliabilities** | Reliability code 2. Reliable with restriction. |
| **Remarks for Data Reliability** | The data are obtained by a recognized fugacity calculation method. Data are considered reliable with restriction because this method does not allow for biodegradation or metabolism. |
| **References** | Mackay D., A.DiGuardo, S.Paterson, G.Kicsi and C.E.Cowan (1996a, 1996b) Assessing the fate of new and existing chemicals: a five-stage process & Evaluating the fate of a variety of types of chemicals using the EQC model. Env. Tox.& Chem., 15(9), 1618-1637. |

# 3 ECOTOXICITY

## 3.1 Acute Toxicity to Fish

| | |
|---|---|
| **Substance Name** | Methyl eugenol (surrogate for estragole) |
| **CAS No.** | 93-15-2 |
| **Remarks for Substance** | Assay: >97% |
| **Test Type** | Experimental |
| **GLP** | No |
| **Year** | 1975 |
| **Species/Strain/Supplier** | Fish/Rainbow trout |
| **Exposure Period** | 96 hour |
| **Remarks for Test Conditions** | Ten fish were used. Each material tested at 5 concentrations. Control groups conducted concurrently. The fish were observed for 96 hours. The fish were placed in bioassay vessels containing reconstituted water, and the test material in acetone was added to the vessels. Ten fish per concentration were used, and each material was tested at five concentrations. Control groups of fish in untreated water and in water to which acetone only was added were observed concurrently. The fish were observed for 96 hours and all deaths and/or behavioral reactions were recorded. The concentration of dissolved oxygen was measured in all solutions in which deaths occurred to be sure the test water contained sufficient oxygen: dissolved oxygen concentrations above 4 mg/liter (4ppm for the warm-water fish (bluegills) or above 5 mg/liter for cold-water fish (rainbow trout) were considered adequate. The median lethal concentrations (LC50) of the test materials were calculated by Litchfield and Wilcoxon method. |
| **Nominal concentrations as mg/L** | 3.2-10 mg/L |
| **Endpoint value** | 6 mg/L 95% C.I. (4.9-7.2) |
| **Reference substances (if used)** | Toxaphene |
| **Conclusion remarks** | The authors concluded that estragole was of a low order of toxicity to fish. 95% confidence level was 4.9-7.2 ppm in rainbow trout. Concentration range was 3.2-10.0 ppm in rainbow trout. With higher doses trout became quiescent and flaccid, swimming or lying on their sides, with slow respiration. Dark discoloration of the integument was also observed. |

| | |
|---|---|
| **Data Qualities Reliabilities** | Reliability code 1. Reliable without restriction. |
| **Remarks for Data Reliability** | Code 1. Comparable to guideline study. |
| **Reference** | Beroza M., Inscoe M., Schwartz P., Kepliknger M. and Mastri C. (1975) Toxicology and Applied Pharmacology 31, 421-429. |

| | |
|---|---|
| **Substance Name** | Methyl eugenol (surrogate for estragole) |
| **CAS No.** | 93-15-2 |
| **Remarks for Substance** | Assay: >97% |
| **Test Type** | Experimental |
| **GLP** | No |
| **Year** | 1975 |
| **Species/Strain/Supplier** | Fish/Bluegill sunfish |
| **Exposure Period** | 96 hour |
| **Remarks for Test Conditions** | Ten fish were used. Each material tested at 5 concentrations. Control groups conducted concurrently. The fish were observed for 96 hours. The fish were placed in bioassay vessels containing reconstituted water, and the test material in acetone was added to the vessels. Ten fish per concentration were used, and each material was tested at five concentrations. Control groups of fish in untreated water and in water to which acetone only was added were observed concurrently. The fish were observed for 96 hours and all deaths and/or behavioral reactions were recorded. The concentration of dissolved oxygen was measured in all solutions in which deaths occurred to be sure the test water contained sufficient oxygen: dissolved oxygen concentrations above 4 mg/liter (4ppm for the warm-water fish (bluegills) or above 5 mg/liter for cold-water fish (rainbow trout) were considered adequate. The median lethal concentrations (LC50) of the test materials were calculated by Litchfield and Wilcoxon method. |
| **Nominal concentrations as mg/L** | 3.2-10 mg/L |
| **Endpoint value** | 8.1 mg/L 95% C.I. (7.4-9.0) |
| **Reference substances (if used)** | Toxaphene |
| **Conclusion remarks** | The authors concluded that estragole was of a low order of toxicity to fish. Calculated LC50 95% confidence limits were 7.4-9.0 ppm in bluegill sunfish. Concentration range was 3.2-10.0 ppm. With higher doses Bluegill sunfish became quiescent and flaccid, swimming or lying on their sides, with slow respiration. Clinical signs at 6 mg/L and greater. |

| | |
|---|---|
| **Data Qualities Reliabilities** | Reliability code 1. Reliable without restriction. |
| **Remarks for Data Reliability** | Code 1. Comparable to guideline study. |
| **Reference** | Beroza M., Inscoe M., Schwartz P., Kepliknger M. and Mastri C. (1975) Toxicology and Applied Pharmacology 31, 421-429. |

| | |
|---|---|
| **Substance Name** | Estragole |
| **CAS No.** | 140-67-0 |
| **Method/guideline** | ECOSAR |
| **Test Type** | Calculated |
| **Species/Strain/Supplier** | Fish |
| **Exposure Period** | 96 hours |
| **Remarks for Test Conditions** | Based on: log KOW = 3.47 and water solubility = 178 mg/L at 25 °C. |
| **Endpoint value** | LC50 = 4.561 mg/L |
| **Data Qualities Reliabilities** | Reliability code 4. Not assignable. |
| **Remarks for Data Reliability** | Code 4. Calculated. |
| **Reference** | ECOSAR EPI Suite (2000) U S Environmental Protection Agency. |

## 3.2 Acute Toxicity to Aquatic Invertebrates

| | |
|---|---|
| **Substance Name** | Estragole |
| **CAS No.** | 140-67-0 |
| **Remarks for Substance** | Chemical Assay: 98.9% |
| **Method/guideline** | OECD 202 |
| **Test Type** | Experimental |
| **GLP** | Yes |
| **Year** | 2003 |
| **Analytical procedures** | HPLC/UV detector |
| **Species/Strain** | Daphnia magna/Aquatic Biosystems, Inc. |
| **Test details** | 48 hrs. |
| **Remarks for Test Conditions** | Juvenile daphnids (<24 hours old) (10/group) produced from an in-house culture of adults were maintained at the contract laboratory under test conditions for 45 days. During the 48 hours prior to testing, the daphnid culture was maintained in 100% dilution water under static, renewal conditions for 48 hours. There was no mortality during the 48 hours prior to test and the test organisms appeared free of disease, injuries, or abnormalities. The daphnid culture produced young before day 12 and a subsample of adults produced on average, more than 3 young per day during the 7days prior to the beginning of the test. The test substance was provided via an intermittent flow proportional diluter. Ten daphnid were randomly selected for each replicate test. Tests were performed at 5 nominal concentrations. During the 48-hr test, daphnid were exposed to 16 hours of light and 8 hours of darkness. Mortality, immobility, and sub-lethal effects were determined visually at 0, 24, and 48 hours. Test temperature was maintained at 19.5-20.7 $_{o}$C |
| **Nominal concentrations as mg/L** | 0, 6.2, 10, 18, 29, 48, and 80 mg/L |
| **Measured concentrations as mg/L** | 0, 4.73, 7.58, 12.9, 34.5, and 56.9 mg/L |
| **Unit** | mg/L |
| **EC50, EL50, LC0, at 24,48 hours** | 48-hr EC50=2.65 mg/Land 48 hr LC50=3.11 mg/L; NOEC 1.14 mg/L |
| **Biological observations** | The number of surviving daphnids at 48 hours for duplicate runs at each mean measured concentration was:0 mg/L, 9/9; 4.74 mg/L, 10/10 & 10/10; 7.58 mg/L, 9/10 & 10/10; 12.9 mg/L, 1/10 & 2/10; 20.4, 34.5, and 56.9 mg/L, 0/10 & 0/10. |
| **Control response** | yes |

| | |
|---|---|
| **satisfactory?** | |
| **Appropriate statistical evaluations?** | Probit method (Stephan, 1978) |
| **Remarks fields for results** | The measured concentrations after 24 and 48 hours were 70-76% of the nominal concentrations, with the concentration being held steady throughout the test period. The respective ranges for conductivity, pH, dissolved oxygen, and temperature were: 550 umhos/cm, 7.3-7.5, 7.8-8.9 mg/L, and 19.5-20.7C, respectively. |
| **Conclusion remarks** | The acute 48-hour EC50 and LC50 for estragole in Daphnia magna under semi-static conditions were 8.87 and 10.5 mg/L, respectively. The NOEC for estragole in Daphnid magna is 4.73 mg/L |
| **Reliabilities** | Reliability Code No. 1. Reliable without restriction. |
| **Remarks for Data Reliability** | The data are obtained by a recognized guideline method and are consistent with chemical structure. |
| **References** | Ward T. (2003) Acute toxicity test with estragole and the Daphnid, Daphnia magna. Study No. 2504-FF. Private communication to FFHPVC. Unpublished Report. |

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| **Substance Name** | Estragole |
| **CAS No.** | 140-67-0 |
| **Remarks for Substance** | Test substance was estragon oil (tarragon oil). Typical composition of estragon oil is (70-88% estragole). |
| **Method/guideline** | OECD Guideline 202-I |
| **Test Type** | Experimental |
| **GLP** | Yes |
| **Year** | 2001 |
| **Species/Strain/Supplier** | *Daphnia magna/Straus* |
| **Test Details** | 48 hours |
| **Remarks for Test Conditions** | Groups of 20 Daphnia magna (Karlsruhe, GDR)(5/1ml test volume) were exposed to test concentrations of 0, 0 (acetone solvent), 3.8,7.5,15.0, 30.0, or 60.0 mg/L of estragon oil for 48 hours. Solution temperature and pH were maintained at 20-20.5 C and 7.98. Invertebrates were held for 16 hours in daylight followed by 8 hours of dark. The conductivity of the water was 0.4 to 1.5 uS/cm and water hardness was 200 mg/L. Daphnia (2-24 hrs. old) were decanted into 25 ml glass beakers, each containing 10 ml of test solution with the test substance in various concentrations. Test solutions were prepared by emulsification of the test substance in water with acetone. There were 5 Daphnia per beaker and 5 beakers per each concentration. Test conditions consisted of a 16 hr./8 hr. light/dark cycle, a light intensity of 200 lx, oxygen concentration |

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| | of 8.3-8.6 and temperature of 20.0-20.5 degrees Celsius. The Daphnia are examined for mobility after 24 and 48 hours. Daphnia which showed no reaction after 15 seconds were considered immobile. The pH, oxygen content and temperature were measured at the beginning and end of the test. Probit analysis was performed to determine the EC50. |
| **Nominal concentrations as mg/L** | 0,3.8,7.5,15.0, 30.0, or 60.0 |
| **Unit** | mg/L |
| **EC50, EL50, LC0, at 24,48 hours** | EC50 = 30.5mg/l (95% CI, 13.3-48 mg/L) |
| **Biological observations** | No reduction in swimming mobility was observed at 0, 3.8, 7.5 or 15 mg/L at 3, 24, or 48 hours. At 30.0 mg/L reduction in swimming mobility was reported for 5/20, 5/20, 8/20 at 3, 24, or 48 hours, respectively.<br>3.8, 7.5 and 15 mg/L- No effect on swimming capacity after 48 hours<br>30.0 mg/L- Statistically significantly reduction in the swimming capacity was observed.<br>60 mg/L- 100% reduction in swimming capacity after 48 hours |
| **Control response satisfactory?** | Yes |
| **Appropriate statistical evaluations?** | Probit Analysis |
| **Remarks fields for results** | Measurement of pH, Oxygen concentration, and temperature at 0 and 48 hours revealed no significant change (7.69-8.02) in pH, O2 concentration (8.3-8.6), or temperature (20 to 20.3C) |
| **Conclusion remarks** | The EC50 for *Daphnia magna* in a static immobilization study was 30.5 mg/L |
| **Data Qualities Reliabilities** | Reliability code 1. Reliable without restriction. |
| **Data Reliability Remarks** | Code 1. Guideline study. |
| **Reference** | Barth M. and Winkler, J (2001) Testing for acute toxicity of estragon oil (*Artemisia dracunculus L.)* in Daphne - *Daphnia magna*. Unpublished report. |

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| **Substance Name** | Estragole |
| **CAS No.** | 140-67-0 |
| **Method/guideline** | ECOSAR |
| **Test Type** | Calculated |

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|---|---|
| **Species/Strain/Supplier** | *Daphnia magna* |
| **Test Details** | 48 hours |
| **Remarks for Test Conditions** | Based on: log KOW = 3.47 and water solubility = 178 mg/L at 25 C. |
| **Unit** | mg/L |
| **EC50, EL50, LC0, at 24,48 hours** | LC50 = 5.410 mg/L |
| **Data Qualities Reliabilities** | Reliability code 4. Not assignable. |
| **Data Reliability Remarks** | Code 4. Calculated. |
| **Reference** | ECOSAR EPI Suite (2000) U S Environmental Protection Agency. |

## 3.3 Acute Toxicity to Aquatic Plants

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|---|---|
| **Substance Name** | Estragole |
| **CAS No.** | 140-67-0 |
| **Remarks for Substance** | Assay: 98.9% |
| **Method/guideline** | OECD 201 Guideline |
| **Test Type** | Experimental |
| **GLP** | Yes |
| **Year** | 2003 |
| **Species/Strain/Supplier** | Green algae/Selenastrum capricornutum/UTEX 1648 |
| **Exposure period (duration)** | 72 hrs |
| **Analytical monitoring** | HPLC/UV detector |
| **Remarks for Test Conditions** | Green Algae/Selenastrum capricornutum/U. of Texas was maintained at test conditions for 14 days prior to the test. The culture was growing in at least 2 subcultures prior to the initiation of the test. In a range finding test, the number of cells/mL was 76% of controls at 0.01 mg/L, >100% of controls at 0.10 mg/L, 51% at 1.0 mg/L, and <3% at 10 and 100 mg/L after three days. In the definitive test, algae was treated with nominal concentrations of 0, 0.13, 0.25, 0.50, 1.0, 2.0 and 4.0 mg/L for 72 hours. pH was adjusted to 7.5 and solutions were exposed for 24 hours of light of intensity, 400 foot candles. The number of algal cells/mL as well as relative size, cell shapes, color, adherence and aggregation of cells was determined. At 24, 48, and 72 hours 3 treatment and 6 control vessels were sacrificed to determine the number of algal cells/mL. |

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| | Concentrations were determined by HPLC. |
| **Nominal concentrations as mg/L** | 0, 0.13, 0.25, 0.50, 1.0, 2.0 and 4.0 mg/L |
| **Measured concentrations as mg/L** | Initial mean measured concentrations 0, 0.118, 0.223, 0.434, 0.875, 1.79, and 3.39 mg/L; Final measured were 85-91% of nominal concentrations |
| **Unit** | mg/L |
| **NOEC, LOEC or NOEL, LOEL** | 72 hr EC50=2.81 mg/L based on average specific growth rate; 72-hr EC50=1.35 mg/L calculated using the number of cells/mL; 72-hr EC50= 1.01 mg/L using the area under the growth curve. The 72-hr NOEC=0.118 mg/L based on number of cells/mL |
| **Biological observations** | Control algal populations grew at an acceptable rate (220,000 cells/ml) after 72 hours. Incubation temperatures were in the range from 23.4 to 23.6 C over the 72 hours and pH was unchanged by the test substance. At the conclusion of the test, samples of test media from each test vessel with maximal growth inhibition were combined with fresh media. After 48 hours incubation the number of cells increased from 410 cells/mL to 230,000 cells/mL at 3.39 mg/L suggesting that the toxic effects were algistatic. |
| **Appropriate statistical evaluations?** | EC50 values determined by weighted least squares non-linear regression (Bruce and Versteeg, 1992); NOEC was determined using a one-way analysis of variance (ANOVA) and Bonferroni's test (Gulley et al. 1990) |
| **Conclusion remarks** | The acute toxicity of estragole measured as a 50% decrease in growth and reproduction of freshwater algae was estimated to be 72 hr EC50=2.81 mg/L based on average specific growth rate; 72-hr EC50=1.35 mg/L calculated using the number of cells/mL; 72-hr EC50= 1.01 mg/L using the area under the growth curve. The 72-hr NOEC=0.118 mg/L based on number of cells/mL |
| **Reliabilities** | Reliabitiy code 1. Reliable without restrictions. |
| **Remarks for Data Reliability** | OECD 201 Guideline study |
| **References** | Boeri R.L.. (2003) The growth and reproduction toxicity test with estragole and freshwater alga, Selenastrum capricornutum. OECD 201. Study No. 2503-FF. Private Communication to FFHPVC. Unpublished Report. |

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|---|---|
| **Substance Name** | Estragole |
| **CAS No.** | 140-67-0 |
| **Method/guideline** | ECOSAR |
| **Test Type** | Calculated |
| **Species/Strain/Supplier** | Green algae |

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|---|---|
| **Exposure Period** | 96 hour |
| **Remarks for Test Conditions** | Based on: log KOW = 3.47 and water solubility = 178 mg/L at 25 °C. |
| **Endpoint Value** | EC50 = 3.681mg/L |
| **Data Qualities Reliabilities** | Reliability code 4. Not assignable. |
| **Remarks for Data Reliability** | Code 4. Calculated. |
| **Reference** | ECOSAR EPI Suite (2000) U S Environmental Protection Agency. |

# 4 HUMAN HEALTH TOXICITY

## 4.1 Acute Toxicity

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|---|---|
| **Substance Name** | Estragole (>95%) |
| **CAS No.** | 140-67-0 |
| **Method/guideline** | Litchfield and Wilcoxon, 1949 |
| **Test Type** | Oral LD50 |
| **GLP** | No |
| **Year** | 1964 |
| **Species/strain** | Rat/Osborne Mendel |
| **Sex** | Male and Female |
| **# of animals per sex per dose** | 5 |
| **Vehicle** | None |
| **Route of Administration** | Oral-Gavage |
| **Remarks for Test Conditions** | The test material was administered to 5 male and 5 female Osborne-Mendel rats per dose. Animals were fasted for 18 hours prior to dosing. All doses were given by intubation. Observations for two weeks included mortality and/or systemic effects. LD50 results were calculated using Litchfield-Wilcoxon (1949). |
| **Value LD50 or LC50 with confidence limits** | 1820 mg/kg bw 95% confidence limits = 1670-1980 mg/kg bw. |
| **Number of deaths at each dose level** | Not given |
| **Remarks for Results** | Death from 4 hours to 8 days. Toxic signs included depression, coma, rough fur, wet posterior and porpyrin-like deposits around eye reported as toxic sign. No necropsy performed. |
| **Conclusion remarks** | The oral LD50 was calculated to be1820 mg/kg bw with 95% confidence limits = 1670-1980 mg/kg bw. |
| **Data Qualities Reliabilities** | Reliability code 2. Reliable with restriction. |
| **Remarks for Data Reliability** | Code 2. Basic data given: comparable to guidelines/standards. |
| **References** | Jenner P.M., Hagan E.C., Taylor J.M., Cook E.L. and Fitzhugh O.G. (1964) Food flavorings and compounds of related structure I. Acute oral toxicity. Food and Cosmetics Toxicology, 2(3), 327-343. |

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|---|---|
| **Substance Name** | Estragole |
| **CAS No.** | 140-67-0 |
| **Method/guideline** | Not given |
| **Test Type** | Oral LD50 |
| **GLP** | No |
| **Year** | 1972 |
| **Species/strain** | Rabbit/New Zealand White |
| **Sex** | Not reported |
| **Number of animals per sex per dose** | 10 |
| **Vehicle** | None |
| **Route of Administration** | Dermal |
| **Remarks for Test Conditions** | Ten New Zealand white rabbits were administered the test substance on their clipped abraded abdominal skin. Observations made for mortality and toxic effects. |
| **Value LD50 or LC50 with confidence limits** | Greater than 5000 mg/kg bw |
| **Number of deaths at each dose level** | 0/10 deaths |
| **Conclusion Remarks** | The dermal LD50 was reported to be greater than 5000 mg/kg bw. |
| **Data Qualities Reliabilities** | Reliability code 2. Reliable with restriction. |
| **Remarks for Data Reliability** | Code 2. Basic data given: comparable to guidelines/standards. |
| **References** | Moreno O. (1972a) Acute dermal toxicity of estragole in rabbits. Unpublished report to RIFM. |

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|---|---|
| **Substance Name** | Estragole (assay:>95%) |
| **CAS No.** | 140-67-0 |
| **Method/Guideline** | Litchfield and Wilcoxon, 1949 |
| **Test Type** | Oral LD50 |
| **GLP** | No |
| **Year** | 1964 |
| **Species/strain** | Mouse |

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|---|---|
| **Sex** | Not reported |
| **Vehicle** | None |
| **Route of Administration** | Oral-Gavage |
| **Remarks for Test Conditions** | Oral doses of test substance given to mice on full stomachs. Doses administered *via* intubation. Mice observed for two weeks. |
| **Value LD50 or LC50 with confidence limits** | 1250 mg/kg bw 95% confidence limits = 812-1920 mg/kg bw |
| **Number of deaths at each dose level** | Not given |
| **Remarks for Results** | Death from 1 hour to 4 days. Toxic signs included depression and coma at higher doses. |
| **Conclusion Remarks** | The oral LD50 was calculated to be1250 mg/kg bw with 95% confidence limits = 812-1920 mg/kg bw. |
| **Data Qualities Reliabilities** | Reliability code 2. Reliable with restriction. |
| **Remarks for Data Reliability** | Code 2. Basic data given: comparable to guidelines/standards. |
| **References** | Jenner P.M., Hagan E.C., Taylor J.M., Cook E.L. and Fitzhugh O.G. (1964) Food flavorings and compounds of related structure I. Acute oral toxicity. Food and Cosmetics Toxicology, 2(3), 327-343. |

| | |
|---|---|
| **Substance Name** | Estragole |
| **CAS No.** | 140-67-0 |
| **Method/Guideline** | Not given |
| **Test Type** | Oral LD50 |
| **GLP** | No |
| **Year** | 1972 |
| **Species/strain** | Rat/Wistar |
| **Sex** | Male |
| **Number of animals per sex per dose** | 10 |
| **Vehicle** | None |
| **Route of Administration** | Oral |
| **Remarks for Test Conditions** | Ten male albino Wistar rats per group were used. Animals were fasted for a minimum of 16 hours prior to administration of the test material. Animals weighed 200-250 grams. Following dosing the animals received food and water *ad libitum*. Observations for mortality were made at 1 and 6 hours after dosing and daily thereafter for 14 days. Toxic effects were also |

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| | observed. Gross necropsies were performed on all survivors. |
| **Value LD50 or LC50 with confidence limits** | 1230 mg/kg bw 95% Confidence Limits (1080-1380 mg/kg bw) |
| **Number of deaths at each dose level** | 820 mg/kg bw: No observable effects, 1030 mg/kg bw: 2/10 deaths, 1230 mg/kg bw: LD50, 1280 mg/kg bw: 6/10 deaths; 1600 mg/kg bw 9/10 deaths. |
| **Conclusion Remarks** | The oral LD50 was calculated to be 1230 mg/kg bw with confidence limits of 1080-1380 mg/kg bw. |
| **Data Qualities Reliabilities** | Reliability code 2. Reliable with restriction. |
| **Remarks for Data Reliability** | Code 2. Basic data given: comparable to guidelines/standards. |
| **References** | Moreno O. (1972b) Acute oral toxicity of estragole in rats. Unpublished report to RIFM. |

## 4.2 Genetic Toxicity

### 4.2.1 *In vitro* Genotoxicity

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|---|---|
| **Substance Name** | Estragole |
| **CAS No.** | 140-67-0 |
| **Remarks for Substance** | Purity 99.% |
| **Method/guideline** | Ames |
| **Test Type** | Reverse mutation |
| **System of Testing** | Bacterial |
| **GLP** | Ambiguous |
| **Year** | 1982 |
| **Species/Strain** | *Salmonella typhimurium* TA 98, TA 100, TA 1535, and TA 1537 |
| **Metabolic Activation** | With and without rat liver microsome fraction S9 from Aroclor induced rats |
| **Doses/Concentration** | 30-300 micrograms/plate |
| **Statistical Methods** | Student's t test |
| **Remarks for Test Conditions** | The assays with S9 were conducted using the pre-incubation method, while the assays without S-9 were conducted using the plate incorporation method. The positive controls were 9-aminoacridiine (9-AAc) with TA1535 and TA1537 (5 ug/plate) and TA1538 (ug/plate); and 5 ug/plate of benzo[a]pyrene (BP) |

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| | with TA98 and TA100. Tests were performed in duplicate |
| **Results** | Negative |
| **Cytotoxic concentration** | 300 ug/plate |
| **Genotoxic Effects** | None |
| **Appropriate statistical evaluations?** | Yes |
| **Remarks for Results** | Estragole was inactive in *Salmonella* strains TA 1535, TA 1537, TA 98 & TA 100 both in the presence and absence of metabolic activation. |
| **Conclusion Remarks** | No evidence of mutagenicity. |
| **Data Qualities Reliabilities** | Reliability code 2. Reliable with restriction. |
| **Remarks for Data Reliability** | Code 2. Basic data given: comparable to guidelines/standards. |
| **References** | Sekizawa J. and Shibamoto T. (1982) Genotoxicity of safrole-related chemicals in microbial test systems. Mutation Research. 101(1), 127-140. |

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|---|---|
| **Substance Name** | Estragole |
| **CAS No.** | 140-67-0 |
| **Remarks for Substance** | Purity 99.% |
| **Method/guideline** | Ames |
| **Test Type** | Reverse mutation |
| **System of Testing** | Bacterial |
| **GLP** | Ambiguous |
| **Year** | 1982 |
| **Species/Strain** | *Escherichia coli* WP2 uvrA trp- |
| **Metabolic Activation** | With and without rat liver microsome fraction S9 from Aroclor induced rats |
| **Doses/Concentration** | 30-300 micrograms/plate |
| **Statistical Methods** | Student's t test |
| **Remarks for Test Conditions** | Conducted as in Ames except that histidine was replaced with tryptophan. A mutagenicity test was conducted on Escherichia coli WP2 uvr A trp-, using the plate incorporation method in the absence of S9 metabolic activation. A mixture of the test material in dimethyl sulfoxide (DMSO), 100 ul of an overnight culture of the E. coli strains, and 500 ul of sodium phosphate buffer (0.1 M) was added to test tubes that contained 2 ml of top agar supplemented with 0.1 umole of tryptophan. The tube contents were mixed and then poured onto minimal agar plates. |

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| | The plates were incubated for 37oC for 48 – 72 hours, and the tryptophan-independent colonies were scored following the incubation period. The positive control was 0.01 ug/plate of 2-(2-furyl)-3-(5-nitro-2-furyl)acrylamide (AF-2). Tests were performed in duplicate. |
| **Results** | Negative |
| **Cytotoxic concentration** | 300 ug/plate |
| **Genotoxic Effects** | None |
| **Appropriate statistical evaluations?** | Yes |
| **Remarks for Results** | Estragole was inactive in *E. coli* WPR uvrA both in the presence and absence of metabolic activation. |
| **Conclusion Remarks** | No evidence of mutagenicity. |
| **Data Qualities Reliabilities** | Reliability code 2. Reliable with restriction. |
| **Remarks for Data Reliability** | Code 2. Basic data given: comparable to guidelines/standards. |
| **References** | Sekizawa J. and Shibamoto T. (1982) Genotoxicity of safrole-related chemicals in microbial test systems. Mutation Research. 101(1), 127-140. |

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|---|---|
| **Substance Name** | Estragole |
| **CAS No.** | 140-67-0 |
| **Method/guideline** | Ames |
| **Test Type** | Reverse mutation |
| **System of Testing** | Bacterial |
| **GLP** | No |
| **Year** | 1977 |
| **Species/Strain** | *Salmonella typhimurium* TA 98, TA 100, TA 1535, TA 1537, and TA1538 |
| **Metabolic Activation** | None |
| **Doses/Concentration** | 0.2 micromolar or 30 micrograms (calculated based on MW of 148.21) |
| **Statistical Methods** | Not given |
| **Remarks for Test Conditions** | The solvent used was ethanol. This data was taken from an abstract of a French article. |
| **Results** | Negative |
| **Cytotoxic concentration** | Not given |

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|---|---|
| **Genotoxic Effects** | None |
| **Appropriate statistical evaluations?** | None given |
| **Remarks for Results** | Negative |
| **Conclusion Remarks** | No evidence of mutagenicity. |
| **Data Qualities Reliabilities** | Reliability code 2. Reliable with restriction. |
| **Remarks for Data Reliability** | Code 2. Acceptable, well-documented publication/study report, which meets basic scientific principles. |
| **References** | Dorange J. L., Delaforge M. Janiaud P. and Padieu P. (1977) Mutagenicity of the metabolites of the epoxide diol pathway of safrole and analogs. Study on *Salmonella typhimurium*. Societe de Biologie de Dijon, 171(5), 1041-1048. |

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|---|---|
| **Substance Name** | Estragole |
| **CAS No.** | 140-67-0 |
| **Remarks for Substance** | Purity 99% |
| **Method/guideline** | Ames |
| **Test Type** | Reverse mutation |
| **System of Testing** | Bacterial |
| **GLP** | Ambiguous |
| **Year** | 1991 |
| **Species/Strain** | *Salmonella typhimurium* TA 98, TA 100, TA 1535 and TA 1537 |
| **Metabolic Activation** | With and without rat liver microsome fraction S9 from Aroclor induced rats |
| **Doses/Concentration** | 0.06-0.5 microliters/plate (0.06-0.48 micrograms/plate) |
| **Statistical Methods** | Not given |
| **Remarks for Test Conditions** | The solvent used was DMSO. The pre-incubation method was used. A modified Ames assay was conducted by the preincubation method, using Salmonella typhimurium strains strains TA1535, TA1537, TA98 and TA100, in the presence and absence S9 metabolic activation obtained from Aroclor-treated male rats. The test material dissolved in 50 ul dimethyl sulfoxide (DMSO), S9 mix (if desired), and the bacterial strains were preincubated together for 20 minutes at 37oC. Next, 2 ml of soft agar was added and the mixture was poured over 30 ml of minimal glucose agar in a Petri dish. Following incubation at 37oC for 2 hours, the revertants per plate were counted. Assays were run in duplicate. Positive controls not given. |
| **Results** | Negative |

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| **Cytotoxic concentration** | Not given |
| **Genotoxic Effects** | None |
| **Appropriate statistical evaluations?** | None given |
| **Remarks for results** | Negative |
| **Conclusion Remarks** | No evidence of mutagenic activity. |
| **Data Qualities Reliabilities** | Reliability code 2. Reliable with restriction. |
| **Remarks for Data Reliability** | Code 2. Basic data given: comparable to guidelines/standards. |
| **References** | Zani F., Massimo G., Benvenuti S., Bianchi A., Albasini A., Melegari M., Vampa G., Bellotti A., Mazza P. (1991) Studies on the genotoxic properties of essential oils with Bacillus subtilis rec-assay and Salmonella microsome reversion assay. Planta Medica, 57(3), 237-241. |

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|---|---|
| **Substance Name** | Estragole |
| **CAS No.** | 140-67-0 |
| **Method/guideline** | Ames |
| **Test Type** | Ames reverse mutation |
| **System of Testing** | Bacterial |
| **GLP** | Ambiguous |
| **Year** | 1987 |
| **Species/Strain** | *Salmonella typhimurium* TA 97, TA 98, TA 100, TA 1535, and TA 1537 |
| **Metabolic Activation** | Rat liver microsome fraction S9 from Aroclor induced rats |
| **Doses/Concentration** | 1-200 micrograms/ml |
| **Statistical Methods** | Not given |
| **Remarks for Test Conditions** | The pre-incubation method was used. The vehicle was DMSO. |
| **Results** | Negative |
| **Cytotoxic concentration** | Not given |
| **Genotoxic Effects** | None |
| **Appropriate statistical evaluations?** | None given |
| **Remarks for results** | Estragole was inactive in *Salmonella* strains TA 1535, TA 1537, TA 97, TA 98 & TA 100 both in the presence and absence of metabolic activation system. A preincubation modification of the Salmonella/microsome test was conducted in the presence and |

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| | absence of liver S9 from Aroclor-induced male Sprague-Dawley rats or male Syrian hamsters. Strains TA98, TA100, TA1535 and TA1537 and/or TA97 were used. Concurrent solvent and positive controls were run with each trial. The positive controls in the absence of metabolic activation were sodium azide (TA1535 and TA100). 9-aminoacridine (TA97 and TA1537), and 4-nitro-o-phenylenediamine (TA98). The positive control for metabolic activation was 2-aminoanthracene for all strains. Trials were run in duplicate. |
| **Conclusion Remarks** | No evidence of mutagenicity. |
| **Data Qualities Reliabilities** | Reliability code 2. Reliable with restriction. |
| **Remarks for Data Reliability** | Code 2. Basic data given: comparable to guidelines/standards. |
| **References** | Zeiger E, Anderson B., Haworth S. Lawlor T., Mortelmans K. and Speck W. (1987) Salmonella mutagenicity tests: III. Results from testing 255 chemicals. Environmental Mutagenesis 9(9), 1-109. |

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|---|---|
| **Substance Name** | Estragole |
| **CAS No.** | 140-67-0 |
| **Method/guideline** | Ames |
| **Test Type** | Reverse mutation |
| **System of Testing** | Bacterial |
| **GLP** | Ambiguous |
| **Year** | 1982 |
| **Species/Strain** | *Salmonella typhimurium* TA 98, TA 100, TA 1535, TA 1537, and TA1538 |
| **Metabolic Activation** | With and without rat liver microsome fraction S9 from Aroclor induced rats |
| **Doses/Concentration** | 0.05 -50 micrograms/plate |
| **Statistical Methods** | Not given |
| **Remarks for Test Conditions** | An Ames plate incorporation test was conducted with and without metabolic activation in strains TA1535, TA100, TA1537, TA1538 and TA98. The vehicle and negative control was ethanol. Metabolic activation was provided by liver S9 prepared from Aroclor 1254-induced rats. The positive control was 10.0 ug/plate 2-aminoanthracine.<br><br>For strain TA1538, metabolic activation was provided by 3'-phosphoadenosine-5'-phosphosulfate (PAPS) and with and without liver S9 prepared from Aroclor 1254-induced rats. |
| **Results** | No mutagenic effects except a significant increase in the revertants per plate was reported for strain TA1538 in the presence of S-9 and PAPS (3'-phosphoadenosine 5'- |

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| | phosphosulfate) cofactor. |
| **Cytotoxic concentration** | Not given |
| **Genotoxic Effects** | See remarks for results. |
| **Appropriate statistical evaluations?** | None given |
| **Remarks for results** | No mutagenic effects except a significant increase in the revertants per plate was reported for strain TA1538 in the presence of S-9 and PAPS (3'-phosphoadenosine 5'-phosphosulfate) cofactor. The authors proposed that mutagenic response was related to the formation of the sulfate ester of an active metabolite. All other strains of Salmonella typhimurium were not mutagenic in assays using PAPS. |
| **Data Qualities Reliabilities** | Reliability code 2. Reliable with restriction. |
| **Remarks for Data Reliability** | Code 2. Basic data given: comparable to guidelines/standards. |
| **References** | To L.P., Hunt T.P. and Andersen M.E. (1982) Mutagenicity of trans-anethole, estragole, eugenol and safrole in the Ames Salmonella typhimurium assay. Bulletin of Environmental Contamination and Toxicology, 28(6), 647-654. |

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|---|---|
| **Substance Name** | Estragole |
| **CAS No.** | 140-67-0 |
| **Method/guideline** | Ames |
| **Test Type** | Reverse mutation |
| **System of Testing** | Bacterial |
| **GLP** | No |
| **Year** | 1979 |
| **Species/Strain** | *Salmonella typhimurium* TA 98, and TA 100 |
| **Metabolic Activation** | Metabolic activation was provided by hepatic S13 fractions prepared from Aroclor 1254-treated CD rats |
| **Doses/Concentration** | The doses were 5-20 umoles/plate in TA100 and up to 30 umoles/plate in TA98 |
| **Statistical Methods** | Not given |
| **Remarks for Test Conditions** | The vehicle and negative control was ethanol. Positive controls were not included. An Ames test was conducted with and without metabolic activation in strains TA100 and TA98 (provided by Ames). The vehicle and negative control was ethanol. Metabolic activation was provided by hepatic S13 fractions prepared from Aroclor 1254-treated CD rats (Charles River) and an NADPH-generating system |
| **Results** | Equivocal. Very weak activity without metabolic activation in |

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| | TA100. Activity increased in TA100 with activation. No effect was seen in TA98. |
| **Cytotoxic concentration** | Not given |
| **Genotoxic Effects** | Positive in TA100. Negative in TA98. |
| **Appropriate statistical evaluations?** | None given |
| **Remarks for results** | Very weak activity without metabolic activation in TA100. Activity increased in TA100 with activation. No effect was seen in TA98. The doses were 5-20 umoles/plate in TA100 and up to 30 umoles/plate in TA98. The metabolites of estragole (1'-hydroxyestragole and estragole-2',3'-oxide) were positive in strains TA100 and TA1535, but were negative in strain TA98 |
| **Conclusion Remarks** | Equivocal. |
| **Data Qualities Reliabilities** | Reliability code 3. Not reliable. |
| **Remarks for Data Reliability** | Code 3. Does not meet important criteria of current standard methods. |
| **References** | Swanson A.B., Chambliss D.D., Blomquist J.C., Miller E.C. and Miller J.A. (1979) The mutagenicities of safrole, estragole, eugenol, *trans*-anethole, and some of their known or possible metabolites for *Salmonella typhimurium* mutants. Mutation Research, 60(2), 142-153. |

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| **Substance Name** | Estragole |
| **CAS No.** | 140-67-0 |
| **Remarks for Substance** | Purity 99.9% |
| **Method/guideline** | Rec assay performed according to Kada *et al*., 1980 |
| **Test Type** | DNA repair |
| **System of Testing** | Bacterial |
| **GLP** | Ambiguous |
| **Year** | 1982 |
| **Species/Strain** | *Bacillus subtilis* H17 Rec + and M45 Rec - |
| **Metabolic Activation** | Rat liver microsome fraction S9 from Aroclor induced Sprague Dawley rats |
| **Doses/Concentration** | 4 mg/disk |
| **Statistical Methods** | Student's t test |
| **Remarks for Test Conditions** | Zones of killing with both strains (Rec + and Rec -) were measured and the difference between them was taken as the rec effect. Conducted according to Kada *et al*. except that 2 E5 spores used instead of 2 E6 to increase the sensitivity of the |

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| | test. |
| **Results** | Negative |
| **Cytotoxic concentration** | Not given |
| **Genotoxic Effects** | None |
| **Appropriate statistical evaluations?** | None given |
| **Remarks for results** | Negative |
| **Conclusion Remarks** | The test substance did not induce DNA repair. |
| **Data Qualities Reliabilities** | Reliability code 2. Reliable with restriction. |
| **Remarks for Data Reliability** | Code 2. Acceptable, well-documented publication/study report, which meets basic scientific principles. |
| **References** | Sekizawa J. and Shibamoto T. (1982) Genotoxicity of safrole-related chemicals in microbial test systems. Mutation Research. 101(1), 127-140. |

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| **Substance Name** | Estragole |
| **CAS No.** | 140-67-0 |
| **Method/guideline** | UDS |
| **Test Type** | DNA repair |
| **System of Testing** | Mammalian |
| **GLP** | Ambiguous |
| **Year** | 1990 |
| **Species/Strain** | Hepatocytes from Male Fisher 344 rats |
| **Metabolic Activation** | No |
| **Doses/Concentration** | 0.148-1480 mg (10-6 to 10-2 M) |
| **Statistical Methods** | Not given |
| **Remarks for Test Conditions** | Unscheduled DNA synthesis was measured by determining the amount of [3H]thymidine incorporated into hepatocyte nuclear DNA during treatment of the cells with test substance. Alkenylbenzene flavors were tested in the unscheduled DNA synthesis (UDS) assay in freshly isolated hepatocytes from male Fischer 344 rats in primary culture. UDS was measured by determining the amount of [3H]thymidine incorporated into hepatocyte nuclear DNA during treatment of the cells with flavor chemicals in Dimethyl sulfoxide (DMSO). Cell viability was assessed by determining the amount of cytoplasmic lactate dehydrogenase (LDH) leakage into overnight cell cultures. In this case, hepatocyte cultures were treated as for the UDS assay, but omitting [3H]thymidine. Positive control was 2- |

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| | acetylaminofluorene. |
| **Results** | Positive. Dose related increase in UDS. 2.7 times greater than control. |
| **Cytotoxic concentration** | 5 X 10-3 M |
| **Genotoxic Effects** | Positive |
| **Remarks for results** | No UDS observed at concentrations at or above 5 X 10-3 M at which there was significant LDH leakage indicating cytotoxicity. |
| **Data Qualities Reliabilities** | Reliability code 2. Reliable with restriction. |
| **Remarks for Data Reliability** | Code 2. Basic data given: comparable to guidelines/standards. |
| **References** | Howes A.J., Chan V.S.W. and Caldwell J. (1990) Structure-specificity of the genotoxicity of some naturally occurring alkenylbenzenes determined by the unscheduled DNA synthesis assay in rat hepatocytes. Food and Chemical Toxicology, 28(8), 537-542. |

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| **Substance Name** | Estragole |
| **CAS No.** | 140-67-0 |
| **Remarks for Substance** | Purity greater than 99% |
| **Method/guideline** | UDS |
| **Test Type** | DNA repair |
| **System of Testing** | Mammalian |
| **GLP** | Ambiguous |
| **Year** | 1992 |
| **Species/Strain** | Hepatocytes from Male Fisher 344 rats |
| **Metabolic Activation** | No |
| **Doses/Concentration** | 10-4 to 10-3 M (14.8-148 mg) |
| **Statistical Methods** | Not given |
| **Remarks for Test Conditions** | Unscheduled DNA synthesis was measured by determining the amount of [3H]thymidine incorporated into hepatocyte nuclear DNA during treatment of the cells with test substance. A ratio of 1.5 is considered to be a positive response. The ability of the test material to induce unscheduled DNA synthesis in hepatocytes derived from male Fischer 344 rats (155-240 g) was evaluated. Vehicle was DMSO. Positive control was 2-Acetamidofluorene 10(5) M. Nuclear DNA [3H]thymidine incorporation was used as a measure of unscheduled DNA synthesis. Cytotoxicity was assessed by lactate dehydrogenase leakage. |

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| **Results** | Positive. Dose related increase in UDS. 2.68 +/- 0.93 times greater than control at 5 X 10-3 M |
| **Cytotoxic concentration** | 5 X 10-3 M |
| **Genotoxic Effects** | Positive |
| **Appropriate statistical evaluations?** | Not given |
| **Remarks for results** | No UDS observed at concentrations above 5 X 10-3 M at which there was significant LDH leakage indicating cytotoxicity. |
| **Data Qualities Reliabilities** | Reliability code 2. Reliable with restriction. |
| **Remarks for Data Reliability** | Code 2. Basic data given: comparable to guidelines/standards. |
| **References** | Chan V.S.W. and J. Caldwell. (1992) Comparative induction of unscheduled DNA synthesis in cultured rat hepatocytes by allylbenzenes and their 1'-hydroxy metabolites. Food and Chemical Toxicology, 30, 831-836. |

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| **Substance Name** | Estragole |
| **CAS No.** | 140-67-0 |
| **Remarks for Substance** | Purity greater than 99% |
| **Method/guideline** | UDS |
| **Test Type** | DNA repair |
| **System of Testing** | Mammalian |
| **GLP** | Ambiguous |
| **Year** | 1992 |
| **Species/Strain** | Hepatocytes from Wistar rats |
| **Metabolic Activation** | No |
| **Doses/Concentration** | 0.01-10 mM (1.48-1482 mg) |
| **Statistical Methods** | Not given |
| **Remarks for Test Conditions** | Unscheduled DNA synthesis was measured by determining the amount of [3H]thymidine incorporated into hepatocyte nuclear DNA during treatment of the cells with test substance. Fifty hepatocytes per slide from 3 different parallel cultures were evaluated for UDS. Results reconfirmed with independent repeat experiment. Net grain values determined by subtracting the mean of three cytoplasm grain counts from the nuclear grain counts. Cytotoxic effects qualified by determination of necrotic cells. UDS positive cells determined to be percentage of cells with five or more net grains increase over negative controls. The unscheduled DNA synthesis (UDS) assay was conducted in cultures of primary rat hepatocytes using test material dissolved in dimethyl sulfoxide. Hepatocytes were |

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| | isolated from 8-10 week old Wistar rats. Experiment was terminated after 18 hours of culture. Grains were counted with a microscope combined with an Artek counter (Model 982b). Cytotoxic effects were qualified by determination of necrotic cells. |
| **Results** | Positive at all concentrations.<br>0.01 millimolar, 0.1 millimolar, 1 millimolar<br>Lethality:<br>10 millimolar |
| **Cytotoxic concentration** | 1 X 10-2 M |
| **Genotoxic Effects** | Positive |
| **Appropriate statistical evaluations?** | None given |
| **Remarks for results** | Positive. |
| **Data Qualities Reliabilities** | Reliability code 2. Reliable with restriction. |
| **Remarks for Data Reliability** | Code 2. Basic data given: comparable to guidelines/standards. |
| **References** | Muller L. Kasper P., Muller-Tegethoff K. and Petr T. (1994) The genotoxic potential in vitro and in vivo of the allyl benzene etheric oils estragole, basil oil and trans-anethole. Mutation Research, 325(4), 129-136. |

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| **Substance Name** | Estragole |
| **CAS No.** | 140-67-0 |
| **Remarks for Substance** | Purity greater than 99% |
| **Method/guideline** | Chromosomal aberrations in V79 cells |
| **Test Type** | Chromosomal Aberration |
| **System of Testing** | Mammalian |
| **GLP** | Ambiguous |
| **Year** | 1992 |
| **Species/Strain** | V79 cells from Wistar rats |
| **Metabolic Activation** | With and without rat liver microsome fraction S9 from Aroclor induced rats |
| **Doses/Concentration** | 10-5 to 10-3 M (1.48 mg- 148 mg) |
| **Statistical Methods** | Not given |
| **Remarks for Test Conditions** | Chromosomal aberrations determined in V79 cells with and without metabolic activation. Cultures harvested 18 hours after treatment. (2 hour treatment with S9 mix). Test material in dimethyl sulfoxide was tested for the induction of chromosome |

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| | aberrations in V79 cells with and without metabolic activation (rat liver S9) or in co-culture with primary rat hepatocytes. Experiments were terminated after 18 hours of culture. Mitomycin C (MMC) and cyclophosphamide (CP) were used as positive controls. The experiments were run with two cultures in parallel. |
| **Results** | Negative-dose was 10(-5) to 10(-3) molar with and without metabolic activation (rat liver S9) or in co-culture with primary rat hepatocytes. |
| **Genotoxic Effects** | Negative |
| **Appropriate statistical evaluations?** | Chi square distribution |
| **Remarks for results** | Negative |
| **Conclusion Remarks** | Estragole did not induce chromosomal aberrations in V79 cells with and without metabolic activation. |
| **Data Qualities Reliabilities** | Reliability code 2. Reliable with restriction. |
| **Remarks for Data Reliability** | Code 2. Basic data given: comparable to guidelines/standards. |
| **References** | Muller L. Kasper P., Muller-Tegethoff K. and Petr T. (1994) The genotoxic potential *in vitro* and *in vivo* of the allyl benzene etheric oils estragole, basil oil and trans-anethole. Mutation Research, 325(4), 129-136. |

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| **Substance Name** | Estragole |
| **CAS No.** | 140-67-0 |
| **Remarks for Substance** | Purity 99% |
| **Method/guideline** | Rec assay performed according to Mazza *et al*., 1982 |
| **Test Type** | DNA repair |
| **System of Testing** | Bacterial |
| **GLP** | Ambiguous |
| **Year** | 1991 |
| **Species/Strain** | *Bacillus subtilis* PB1652 and PB1791 |
| **Metabolic Activation** | None |
| **Doses/Concentration** | 10-30 microliters (9.6-29 micrograms/plate) |
| **Statistical Methods** | Not given |
| **Remarks for Test Conditions** | A positive DNA damaging activity was assumed when the ratio of the inhibition zone of the rec- mutant and that of the parental rec + strain exceeded the value of 1.2. The test material was applied to a sterile filter paper disk (9 mm diameter) which was placed on the surface of nutrient agar plates seeded with the |

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| | tester strains. After an overnight incubation at 37oC, the diameter of the inhibition zones which formed around the disk, were measured with a Vernier caliper. Methyl methanesulfonate (MMS), mitomycin C (MIT C), adriamicin (ADR) were used as positive controls, while ampicillin (AMP) and chloramphenicol (CAF) as negative controls. |
| **Results** | Positive |
| **Cytotoxic concentration** | Not given |
| **Genotoxic Effects** | Positive |
| **Appropriate statistical evaluations?** | None given |
| **Remarks for Results** | Positive |
| **Data Qualities Reliabilities** | Reliability code 2. Reliable with restriction. |
| **Remarks for Data Reliability** | Code 2. Basic data given: comparable to guidelines/standards. |
| **References** | Zani F., Massimo G., Benvenuti S., Bianchi A., Albasini A., Melegari M., Vampa G., Bellotti A., Mazza P. (1991) Studies on the genotoxic properties of essential oils with Bacillus subtilis rec-assay and Salmonella microsome reversion assay. Planta Medica 57(3), 237-241. |

### 4.2.2 *In vivo* Genotoxicity

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| **Substance Name** | Estragole |
| **CAS No.** | 140-67-0 |
| **Method/guideline** | 32P-post-labelling analysis of DNA adducts |
| **Test Type** | Adduct formation |
| **GLP** | No |
| **Year** | 1984 |
| **Species/Strain** | Mouse/CD-1 |
| **Sex** | Female |
| **Route of Administration** | Intraperitoneal |
| **Doses/Concentration** | 2 or 10 mg/mouse |
| **Exposure Period** | Single dose |
| **Remarks for Test Conditions** | Groups of 3-4 female CD-1 mice were given an intraperitoneal injection of 0, 2 or 10 mg estragole/mouse in 0.1 ml trioctanoin. Twenty-four hours following treatment, mice were killed and livers were collected and frozen at -80 deg C. DNA was isolated from the frozen livers using a rapid solvent-extraction procedure and quantitated spectrophotometrically. DNA was digested and 32P-labelled. Labelled adducts were purified by reversed phase thin layer chromatography and contact transfer to polyethyleneimine-cellulose. Adduct levels (as reactive adduct labelling [RAL]) were determined (adduct spot/normal nucleotidesx600) and covalent binding indices (CBI) were calculated (umol of anethole bound/mol of DNA nucleotides divided by mmol of anethole administered/kg bw). |
| **Genotoxic effects** | Positive |
| **NOEL (C)/ LOEL (C)** | LOEL: 2 mg/kg bw |
| **Remarks for Results** | DNA adducts were detected at both dose levels. |
| **Conclusion Remarks** | Estragole showed binding potential to mouse-liver DNA. |
| **Data Qualities Reliabilities** | Reliability code 2. Reliable with restriction. |
| **Remarks for Data Reliability** | Code 2. Comparable to guideline study with acceptable restrictions. |
| **References** | Randerath, K., Haglund, R.E., Phillips, D.H., and Reddy, M.V. (1984) 32P-Post-labelling analysis of DNA adducts formed in the livers of animals treated with safrole, estragole and other naturally occurring alkenylbenzenes. I. Adult female CD-1 mice. Carcinogenesis 5(12): 1613-1622. |

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| **Substance Name** | Estragole |
| **CAS No.** | 140-67-0 |
| **Method/guideline** | 32P-post-labelling analysis of DNA adducts |
| **Test Type** | Adduct formation |
| **GLP** | No |
| **Year** | 1981 |
| **Species/Strain** | Mouse/B6C3F1 |
| **Sex** | Male and Female |
| **Route of Administration** | Intraperitoneal |
| **Doses/Concentration** | 14 mg/kg bw |
| **Exposure Period** | Single dose |
| **Remarks for Test Conditions** | In a study designed to detect DNA adduct formation of estragole, 9-day old male or female B6C3F1 mice (mean weight, 6g) were given intraperitoneal injections of 0.5 mmol (14 mg/kg) of labeled estragole and sacrificed after 23 hours. |
| **NOEL (C)/ LOEL (C)** | LOEL: 14 mg/kg bw |
| **Genotoxic effects** | Positive |
| **Remarks for Results** | DNA adducts were detected. |
| **Conclusion Remarks** | Estragole showed binding potential to mouse-liver DNA. |
| **Data Qualities Reliabilities** | Reliability code 2. Reliable with restriction. |
| **Remarks for Data Reliability** | Code 2. Comparable to guideline study with acceptable restrictions. |
| **References** | Phillips, D.H., J.A. Miller, E.C. Miller, and B. Adams. (1981) Structures of the DNA adducts formed in mouse liver after administration of the proximate hepatocarcinogen 1'-hydroxyestragole. Cancer Research, 41, 176-186. |

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| **Substance Name** | Estragole |
| **CAS No.** | 140-67-0 |
| **Remarks for Substance** | Purity 98% |
| **Method/guideline** | *in vivo* UDS |
| **Test Type** | DNA repair |
| **GLP** | Ambiguous |

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| **Year** | 1994 |
| **Species/Strain** | Rat/Wistar |
| **Sex** | Male |
| **Route of Administration** | Gavage |
| **Doses/Concentration** | 500, 1,000 or 2,000 mg/kg bw |
| **Exposure Period** | Single dose |
| **Remarks for Test Conditions** | Test material in peanut oil was administered to male Wistar rats at dose levels of 500, 1,000 or 2,000 mg/kg bw. Hepatocytes isolated from sacrificed rats 4 or 12 hours after the single dose. After 18 hours of culture, fifty hepatocytes per slide were evaluated for UDS. Net grain values obtained by subtracting the mean of three cytoplasm grain counts from the nuclear grain counts. Cytotoxic effects determined by the number of necrotic cells. Cells considered positive for UDS if percentage of cells with five or more net grains increased over the negative concurrent control values. |
| **Genotoxic effects** | 500 mg/kg bw- weak effect; 1,000 mg/kg weak effect; 2,000 mg/kg clear positive effect at this dose level. No difference between cells isolated at 4 hours and those isolated at 12 hours. |
| **NOEL (C)/ LOEL (C)** | LOEL: 500 mg/kg bw |
| **Appropriate statistical evaluations?** | None given |
| **Remarks for Results** | Only a very slight increase in net grain values reported for the 500 and 1000 mg/kg bw dose levels. The highest dose levels produced clear increases. |
| **Conclusion Remarks** | The authors characterize the results seen at the two lowest dose levels as being very slight increases and given the lack of appropriate statistical analyses, these results are considered questionable. |
| **Data Qualities Reliabilities** | Reliability code 2. Reliable with restriction. |
| **Remarks for Data Reliability** | Code 2. Basic data given: comparable to guidelines/standards. |
| **References** | Muller L. Kasper P., Muller-Tegethoff K. and Petr T. (1994) The genotoxic potential in vitro and in vivo of the allyl benzene etheric oils estragole, basil oil and trans-anethole. Mutation Research, 325(4), 129-136. |

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| **Substance Name** | Estragole |
| **CAS No.** | 140-67-0 |
| **Method/guideline** | 32P-post-labelling analysis of DNA adducts |
| **Test Type** | Adduct formation |

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| **GLP** | No |
| **Year** | 1984 |
| **Species/Strain** | Mouse/B6C3F1 |
| **Sex** | Male |
| **Route of Administration** | Intraperitoneal |
| **Doses/Concentration** | 0.25, 0.5, 1.0, and 3.0 mmol |
| **Exposure Period** | 23, 29 or 43 days |
| **Remarks for Test Conditions** | 32P-post-labelling analysis was used to detect test material-DNA adducts in livers of treated mice. B6C3F1 male mice received 0.25, 0.5, 1.0 and 3.0 umol of test material on days 1, 8, 15 and 22, respectively, after birth. Groups of 3 mice were killed for analysis on days 23, 29 and 43 (i.e. 1, 7, and 21 days after the final injection) and the livers removed and weighed. Vehicle was trioctanoin. |
| **Genotoxic effects** | Positive |
| **Remarks for Results** | DNA adducts were detected. |
| **Conclusion Remarks** | Estragole showed binding potential to mouse-liver DNA. |
| **Data Qualities Reliabilities** | Reliability code 2. Reliable with restriction. |
| **Remarks for Data Reliability** | Code 2. Comparable to guideline study with acceptable restrictions. |
| **References** | Phillips D.H., Reddy M.V. and Randerath K. (1984) 32P-Post-labelling analysis of DNA adducts formed in the livers of animals treated with safrole, estragole and other naturally occurring alkenylbenzenes. II. Newborn male B6C3F1 mice. Carcinogenesis, 5(12), 1623-1628. |

## 4.3 Repeated Dose Toxicity

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| **Substance Name** | Methyl eugenol (surrogate for estragole) |
| **CAS No.** | 93-15-2 |
| **Remarks for Substance** | Data is for structurally related alkoxybenzene derivative, methyl eugenol. Purity greater than 98% |
| **Method/guideline** | OECD Guideline 407 “Repeated Dose 28-day Oral Toxicity Study in Rodents) |
| **GLP** | Yes |
| **Year** | 2004 |

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| **Species/strain** | Rat/F344/N |
| **Sex** | Male and Female |
| **Route of Administration** | Oral-Microencapsulated in the diet |
| **Doses/concentration Levels** | 0, 1, 5, or 50 mg/kg bw/d; |
| **Exposure Period** | 28 days |
| **Frequency of Treatment** | Continuous |
| **Control Group** | Yes |
| **Post Exposure** | None |
| **Remarks for Test Conditions** | The study was designed to investigate the systemic toxicity of the test material. It is based on the recommendations of the OECD Guidelines for Testing of Chemicals No. 407 "Repeated Dose 28 Day Oral Toxicity Study in Rodents" (Adopted 27 July 1995)<br><br>Dietary<br><br>The test material was administered continuously throughout the treatment period by dietary admixture to three groups each of ten male and ten female Fischer 344 F344/NHsd strain rats, for twenty-eight consecutive days, at dose levels of 1, 5 and 50 mg/kg/day. Two control groups each of five males and five females was treated with untreated diet only or untreated diet plus microencapsulated matrix (50 mg/kg bw/day).<br><br>*Gavage.*<br><br>The test material was administered by gavage to ten male and ten female Fischer 344 F344/NHsd strain rats, for twenty-eight consecutive days, at a dose level of 50 mg/kg/day. A control group of five males and five females was dosed with vehicle alone (distilled water). Clinical signs, bodyweight development, urine samples, food and water consumption were monitored during the study. Hematology, urinalysis and blood chemistry were evaluated for all animals at the end of the study. Blood samples of test and control group animals were taken once prior to the start of treatment and again on Day 28. Plasma was separated and stored at approximately -20°C prior to dispatch to Dr Paul Carmichael, Imperial Collage, London. Biochemical studies on DNA and protein adduct formation and PCNA cell proliferation studies were performed on liver and forestomach tissues<br><br>All animals were subjected to a gross necropsy examination and at necropsy blood samples were collected, allowed to clot and serum was then separated and stored at approximately -20°C prior to despatch to Dr Paul Carmichael, Imperial College, London.<br><br>Preliminary histopathological evaluation of the stomach and |

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| | liver from all animals was performed. Additional samples of the liver and stomach from all animals were stored in liquid nitrogen prior to despatched to Paul Ellis and Dr Paul Carmichael, Imperial College, London. |
| **NOAEL(NOEL)** | 50 mg/kg bw/d in the diet |
| **LOAEL(LOEL)** | Not determined |
| **Toxic Response/effects by Dose Level** | See remarks for results. |
| **Appropriate statistical evaluations?** | Yes |
| **Remarks for results** | There were no unscheduled deaths during the study. One Dietary female treated with 50 mg/kg/day developed a damaged tail from Day 14 onwards. Four high dose gavage males showed fur loss from Day 4 onwards and a further two high dose gavage males showed fur loss from Day 11 onwards. High dose gavage males showed a slight reduction in bodyweight gain during Week 1 of treatment. There was no effect on bodyweight in any group of males and female by either the dietary or gavage route of administration. There were no differences between food intake or food efficiency uptake for any of the treated groups compared to controls. Haematological examination, blood chemical determinations and urine analysis revealed a slight increase in cholesterol in gavage males at the 50 mg/kg bw/d per day dose level.<br><br>Dietary animals treated with 1, 5 or 50 mg/kg/day showed a slight reduction in liver weight relative to bodyweight and absolute weight (males only).There was no treatment-related organ weight changes detected in gavage animals treated with 50 mg/kg/day. One dietary female treated with 1 mg/kg/day showed small nodules on the median lobe attached to the diaphragm of the liver. Three high dose gavage males showed fur loss and one high dose dietary female had a damaged tail. There were no further macroscopic abnormalities detected. There were no treatment-related changes detected. $P^{32}$-postlabeling experiments indicate that at detection limits of $1/10^9$ adducts, no methyl eugenol-DNA adduct are detected at 1 mg/kg bw/day and there is equivocal evidence of adduct formation at 5 mg/kg bw/d |
| **Conclusion Remarks** | |
| **Data Qualities Reliabilities** | Reliability code 1. Reliable without restriction. |
| **Remarks for Data Reliability** | Code 1. Guideline study. |

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| **References** | Jones L. (2003) Twenty-eight day repeated dose oral (dietary and gavage) toxicity study in the rat. SPL Project No. 1834/002. Unpublished report to FEMA. |

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| **Substance Name** | Estragole |
| **CAS No.** | 140-67-0 |
| **Remarks for Substance** | Purity greater than 98% |
| **Method/guideline** | National Toxicology Program. 90-Day oral toxicity study |
| **GLP** | Yes |
| **Year** | 2005 |
| **Species/strain** | Mouse/B6C3F1 |
| **Sex** | Male and Female |
| **Route of Administration** | Oral-Gavage |
| **Doses/concentration Levels** | 0, 37.5, 75, 150, or 300 mg/kg bw/d for females<br>and 0, 37.5, 75, 150, 300, or 600 mg/kg bw/d for males |
| **Exposure Period** | 93 days |
| **Frequency of Treatment** | Daily (5 days/week) |
| **Control Group** | Yes |
| **Post Exposure** | None |
| **Remarks for Test Conditions** | Groups of 10 female mice each were administered 0, 37.5, 75, 150, or 300 mg/kg bw/d estragole via gavage once per day, five days per week for 93 days. Males were administered an additional dose level of 600 mg/kg bw/d. Animals were housed five per cage and fed ad libitum. Body weights and clinical observations were made weekly and on day 1 and at termination (day 93). At termination, blood was taken for clinical chemistry and haematology determinations and body and organ weights (heart, brain, liver right kidney, right testes, lungs, and thymus) were recorded. Tissues were prepared and histopathological examination was performed on a wide variety of tissues including the oesophagus, rectum, liver, bile ducts, salivary gland, stromal gland, epidydimus, testes, pancreas, haemapoietic system, olfactory epithelium, and kidney. |
| **NOAEL(NOEL)** | 75 mg/kg bw/d |
| **LOAEL(LOEL)** | 150 mg/kg bw/d |
| **Toxic Response/effects by Dose Level** | See remarks for results. |

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| **Appropriate statistical evaluations?** | Yes |
| **Remarks for results** | In male mice, survival was 100% for all dosed groups except for the 600 mg/kg bw/d level. Statistically significant decreases in body weight were recorded for the 300 and 300 mg/kg bw/d dosed groups compared to that of controls. Haematology examinations revealed decreases in erythrocytes and increases in the number of leucocytes, lymphocytes, reticulocytes, and platelets but only at the 300 and 600 mg/kg bw/d dose levels. Organ weight changes included increased relative (to body weight) liver and decreased body weight at 300 and 600 mg/kg bw/d.<br><br>Histopathological examination revealed liver alterations at 300 and 600 mg/kg bw/d including oval cell hyperplasia, hepatocyte hypertrophy, hepatocyte degeneration all of which were described as being minimal or mild in severity.<br><br>Effects in female mice were less pronounced than in males. Survival was 100% for all dosed groups. Statistically significant decreases in body weight were recorded for the 150 and 300 mg/kg bw/d dosed groups compared to that of controls. Haematology examinations revealed decreases in erythrocytes and increases in the number of platelets, leucocytes, lymphocytes, and reticulocytes 150 and 300 mg/kg bw/d. Organ weight changes increased absolute and relative (to body weight) liver weight at 300 mg/kg bw/d.<br><br>Histopathological examination revealed no alterations to the liver or any other organ or tissues at levels up to and including 300 mg/kg bw/d. |
| **Conclusion Remarks** | Based primarily on the histopathologic changes, a NOAEL of 75 mg/kg bw/d and a LOAEL of 150 mg/kg bw/d was reported for the subchronic toxicity of estragole in male and female mice. |
| **Data Qualities Reliabilities** | Reliability code 1. Reliable without restriction. |
| **Remarks for Data Reliability** | Code 1. Guideline study. |
| **References** | National Toxicology Program (NTP) (2005) Toxicology and of estragole in F344/N Rats and B6C3F1 mice. U.S. Dept of Health and Human Services. NIH Publication No., not assigned. |

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| **Substance Name** | Estragole |
| **CAS No.** | 140-67-0 |
| **Remarks for Substance** | Purity greater than 98% |
| **Method/guideline** | National Toxicology Program. 90-Day oral toxicity study |
| **GLP** | Yes |
| **Year** | 2005 |

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| **Species/strain** | Rat/F344/N |
| **Sex** | Male and Female |
| **Route of Administration** | Oral-Gavage |
| **Doses/concentration Levels** | 0, 37.5, 75, 150, 300, or 600 mg/kg bw/d |
| **Exposure Period** | 93 days |
| **Frequency of Treatment** | Daily (5 days/week) |
| **Control Group** | Yes |
| **Post Exposure** | None |
| **Remarks for Test Conditions** | Groups of 10 male and 10 female rats each were administered 0, 37.5, 75, 150, 300, or 600 mg/kg bw/d estragole via gavage once per day, five days per week for 93 days. Animals were housed five per cage and fed ad libitum. Body weights and clinical observations were made weekly and on day 1 and at termination (day 93). At termination, blood was taken for clinical chemistry and haematology determinations and body and organ weights (heart, brain, liver right kidney, right testes, lungs, and thymus) were recorded. Tissues were prepared and histopathological examination was performed on a wide variety of tissues including the oesophagus, rectum, liver, bile ducts, salivary gland, stromal gland, epididymus, testes, pancreas, hemapoietic system, olfactory epithelium, and kidney. |
| **NOAEL(NOEL)** | Undetermined |
| **LOAEL(LOEL)** | 37.5 mg/kg bw/d |
| **Toxic Response/effects by Dose Level** | See remarks for results. |
| **Appropriate statistical evaluations?** | Yes |
| **Remarks for results** | In male rats, survival was 100% for all dosed groups. Statistically significant decreases in body weight were recorded for the 300 and 300 mg/kg bw/d dosed groups compared to that of controls. Clinical chemistry changes were limited mainly to the 300 and 600 mg/kg bw/d groups. At 300 and 600 mg/kg bw/d increased levels of blood urea nitrogen, total protein, alanine aminotransferase, bile acid salts, and total iron binding capacity were reported. Additionally at 600 mg/kg bw/d, increases in alkaline phosphatase, bile acids/salts, and succinate dehydrogenase were reported. Haematology examinations revealed decreases in erythrocytes, hemaglobin, hematocrit, mean cell volume and platelet count and increased in the number of leucocytes, lymphocytes, and neutrophils but only at the 300 and 600 mg/kg bw/d dose levels. Organ weight changes included increased absolute and relative (to body weight) liver and kidney weight and decreased body weight and testes weight at 300 and 600 mg/kg bw/d. Absolute and relative heart weight was also increased at 600 mg/kg bw/d |

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| | Histopathological examination revealed liver alterations at 37.5 mg/kg bw/d including bile duct hyperplasia, oval cell hyperplasia, hepatocyte hypertrophy, periportal inflammation al of which were described as being minimal effects. Similar alterations at the 75 mg/kg bw per day dose level, were also described as minimal. The severity of hepatic effects at 150 mg/kg bw/d was reported to be mild while the effects at higher dose levels increased in severity (moderate and marked). At 150 mg/kg bw per day and higher dose levels, males also showed evidence of chronic hepatic inflammation, hepatocellular necrosis, oval cell hyperplasia, and hepatic periportal fibrosis. At 600 mg/kg bw/d, cholangiofibrosis was reported in one animal.<br><br>Effects in female rats were similar to those in males, but the onset and the severity of the effects were less pronounced than in males. Survival was 100% for all dosed groups. Statistically significant decreases in body weight were recorded for the 300 and 300 mg/kg bw/d dosed groups compared to that of controls. The only consistent clinical observation occurred among high dose animals that appeared gaunt during the course of the study. Clinical chemistry changes were limited mainly to the 300 and 600 mg/kg bw/d groups. At 300 and 600 mg/kg bw/d increased levels of creatine kinase, succinate dehydrogenase, alanine aminotransferase, and total iron binding capacity were reported. Decreased serum iron was also reported at the two highest dose levels. Additionally at 600 mg/kg bw/d, increases in creatinine, total protein, and albumin were reported. Haematology examinations revealed decreases in erythrocytes, haemaglobin, haematocrit, mean cell volume, mean cell haemoglobin, and reticulocytes. Increases in the number of platelets, leucocytes, lymphocytes, monocytes, and neutrophils were reported beginning at the 75 mg/kg bw/d dose level. These changes were more pronounced at higher dose levels. Organ weight changes included decreased body weights at 300 and 600 mg/kg bw/d, increased absolute and relative (to body weight) liver at dose levels of 37.5 mg/kg bw/d and higher, increased absolute and relative lung weight at 300 and 600 mg/kg bw/d, increased thymus weights at dose levels of 75 mg/kg bw/d and greater, increased heart and right kidney weight at 600 mg/kg bw/d.<br><br>Histopathological examination revealed liver alterations at mainly beginning at the 75 mg/kg bw per day dose levels. At 37.5 mg/kg bw/d minimal bile duct hyperplasia, oval cell hyperplasia, eosinophilic foci, and sporatic periportal inflammation was reported. At 75 mg/kg bw/d, the same alterations were observed with greater incidence and severity. Also basophilic foci were reported at this dose level. At 150 mg/kg bw/d, additional alterations included histiocytic cell infiltrate, hepatocyte hypertrophy, mixed cell foci, At 300 and 600 mg/kg bw/day the severity of the effects was greater. Cholangiofibrosis was reported in one animal at 600 mg/kg bw/d. |
| **Conclusion Remarks** | Based primarily on the histopathologic changes, a LOAEL of 37.5 mg/kg bw/d was reported for the subchronic toxicity of |

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| | estragole in male and female F344/N rats. |
| **Data Qualities Reliabilities** | Reliability code 1. Reliable without restriction. |
| **Remarks for Data Reliability** | Code 1. Guideline study. |
| **References** | National Toxicology Program (NTP) (2005) Toxicology and of estragole in F344/N Rats and B6C3F1 mice. U.S. Dept of Health and Human Services. NIH Publication No., not assigned. |
| **Substance Name** | Methyl eugenol (surrogate for estragole) |
| **CAS No.** | 93-15-2 |
| **Remarks for Substance** | Data is for structurally related substance, methyl eugenol:Purity greater than 98% |
| **Method/guideline** | National Toxicology Program. 14 week oral toxicity study |
| **GLP** | Yes |
| **Year** | 2000 |
| **Species/strain** | Mouse/B6C3F1 |
| **Sex** | Male and Female |
| **Route of Administration** | Oral-Gavage in 0.5% methyl cellulose |
| **Doses/concentration Levels** | 0, 10, 30, 100, 300 or 1000 mg/kg bw/d |
| **Exposure Period** | 14 weeks |
| **Frequency of Treatment** | Daily (5 days/week) |
| **Control Group** | Yes |
| **Post Exposure** | None |
| **Remarks for Test Conditions** | Groups of 10 female mice each were administered 0, 10, 30, 100, 300, or 1000 mg/kg bw/d methyl eugenol via gavage in 0.5% methyl cellulose once per day, five days per week for 93 days. Animals were housed individually and fed ad libitum. Body weights and clinical observations were made weekly and on day 1 and at termination. At termination, blood was taken for clinical chemistry and haematology determinations and body and organ weights (heart, brain, liver right kidney, right testes, lungs, and thymus) were recorded. Tissues were prepared and histopathological examination was performed on a wide variety of tissues including the oesophagus, rectum, liver, bile ducts, salivary gland, stromal gland, epidydimus, testes, pancreas, haemapoietic system, olfactory epithelium, and kidney. |
| **NOAEL(NOEL)** | 10 mg/kg bw/d |

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| **LOAEL(LOEL)** | 30 mg/kg bw/d based on liver weight increases in males |
| **Toxic Response/effects by Dose Level** | See remarks for results. |
| **Appropriate statistical evaluations?** | Yes |
| **Remarks for results** | In mice, low survival rates were reported at the highest dose level of methyl eugenol in males and females. Mean body weight gains of male and female mice given 300 mg/kg were significantly less than those of the vehicle control. There was a statistical increase ($p<0.05$) in liver weights in male mice dosed with $\geq$30 mg/kg bw/d and in female mice dosed with 300 mg/kg bw/d compared to those of the respective control groups. Increased incidences of cytologic alteration, necrosis, bile duct hyperplasia, and subacute inflammation were observed in the liver of 1,000 mg/kg male mice and 300 mg/kg and greater female mice. A significant increase in testis weight was observed in male mice receiving 100 or 300 mg/kg/d. There were no significant findings at 10 mg/kg bw/d [NTP, 2000]. |
| **Conclusion Remarks** | Based primarily on the liver weight changes in males, a NOAEL of 10 mg/kg bw/d and a LOAEL of 30 mg/kg bw/d was reported for the subchronic toxicity of methyl eugenol in male and female mice. |
| **Data Qualities Reliabilities** | Reliability code 1. Reliable without restriction. |
| **Remarks for Data Reliability** | Code 1. Guideline study. |
| **References** | National Toxciology Program (NTP). (2000) Toxicology and carcinogenesis studies of methyleugenol (CAS No. 93-15-12) in F344/n rats and B6C3F1 mice (gavage studies). DRAFT NTP-TR-491; NIH Publication No. 98-3950. |
| **Substance Name** | Methyl eugenol (surrogate for estragole) |
| **CAS No.** | 93-15-2 |
| **Remarks for Substance** | Data is for structurally related substance, methyl eugenol:Purity greater than 98% |
| **Method/guideline** | National Toxicology Program. 14 week oral toxicity study |
| **GLP** | Yes |
| **Year** | 2000 |
| **Species/strain** | Rat/F344/N |
| **Sex** | Male and Female |
| **Route of Administration** | Oral-Gavage in 0.5% methyl cellulose |

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| **Doses/concentration Levels** | 0, 10, 30, 100, 300 or 1000 mg/kg bw/d |
| **Exposure Period** | 14 weeks |
| **Frequency of Treatment** | Daily (5 days/week) |
| **Control Group** | Yes |
| **Post Exposure** | None |
| **Remarks for Test Conditions** | Groups of 10 female and male rats each were administered 0, 10, 30, 100, 300, or 1000 mg/kg bw/d methyl eugenol via gavage in 0.5% methyl cellulose once per day, five days per week for 14 weeks. Animals were housed individually and fed ad libitum. Body weights and clinical observations were made weekly and on day 1 and at termination. At termination, blood was taken for clinical chemistry and haematology determinations and body and organ weights (heart, brain, liver right kidney, right testes, lungs, and thymus) were recorded. Tissues were prepared and histopathological examination was performed on a wide variety of tissues including the oesophagus, rectum, liver, bile ducts, salivary gland, stromal gland, epidydimus, testes, pancreas, haemapoietic system, olfactory epithelium, and kidney. |
| **NOAEL(NOEL)** | 10 mg/kg bw/d |
| **LOAEL(LOEL)** | 30 mg/kg bw/d based on liver weight increases in males |
| **Toxic Response/effects by Dose Level** | See remarks for results. |
| **Appropriate statistical evaluations?** | Yes |
| **Remarks for results** | The final mean body weight gains of male rats receiving 300 and 1,000 mg/kg bw/d and all the dosed female rats were significantly ($p=0.01$) less than those of the vehicle control. Liver weights in male rats dosed with $\geq$100 mg/kg bw per day and in female rats dosed with $\geq$300 mg/kg bw/d were significantly higher than those in control rats. Relative liver weights of male rats at 30 mg/kg bw per day were increased (14.08 g) compared to the vehicle controls (12.87 g) but not with respect to untreated controls (13.56 g). A significant increase in testis weight was observed in male rats receiving 1,000 mg/kg/d. Haematological examination revealed a decreased mean packed red cell volume in 300 mg/kg/d male rats and in male and female rats receiving 1,000 mg/kg/d. There were also increased platelet counts and increased alanine aminotransferase and sorbitol dehydrogenase activities in male and female rats receiving $\geq$100 mg/kg/d. Additionally, hypoproteinemia, hypoalbuminemia, and increased bile acid concentrations were evident in male and female rats receiving $\geq$300 mg/kg/d. An increase in the incidence of adrenal gland cortical hypertrophy and/or cytoplasmic alteration in the submandibular gland occurred in 100 mg/kg or greater male and female rats. The incidences of atrophy and chronic inflammation (chronic gastritis) of the glandular stomach |

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| | mucosa were significantly increased in male and female rats administered 300 mg/kg or greater, and there was a hepatocellular adenoma in one male rat administered 1,000 mg/kg. There were no significant findings at 10 mg/kg bw/d |
| **Conclusion Remarks** | Based primarily on the liver weight changes in males, a NOAEL of 10 mg/kg bw/d and a LOAEL of 30 mg/kg bw/d was reported for the subchronic toxicity of methyl eugenol in male and female rats. |
| **Data Qualities Reliabilities** | Reliability code 1. Reliable without restriction. |
| **Remarks for Data Reliability** | Code 1. Guideline study. |
| **References** | National Toxciology Program (NTP). (2000) Toxicology and carcinogenesis studies of methyleugenol (CAS No. 93-15-12) in F344/n rats and B6C3F1 mice (gavage studies). DRAFT NTP-TR-491; NIH Publication No. 98-3950. |

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| **Substance Name** | Estragole |
| **CAS No.** | 140-67-0 |
| **Remarks for Substance** | Purity greater than 95% |
| **Method/guideline** | Carcinogenicity Study |
| **GLP** | No |
| **Year** | 1983 |
| **Species/strain** | Mice/CD-1 |
| **Sex** | Male and Female |
| **Route of Administration** | Gavage |
| **Doses/concentration Levels** | 0, 370 mg/kg bw |
| **Exposure Period** | Five weeks |
| **Frequency of Treatment** | Twice a week for 10 doses |
| **Control Group** | Yes |
| **Post Exposure** | 13 months |
| **Remarks for Test Conditions** | Male (55) and female (49) CD-1 mice were administered 370 mg/kg of estragole by gavage twice a week for ten doses beginning at 4 days of age. The mice were weaned at 35 days of age following the last intubation. |
| **NOAEL(NOEL)** | Not determined |
| **LOAEL(LOEL)** | 370 mg/kg bw |

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| **Toxic Response/effects by Dose Level** | See remarks for results |
| **Appropriate statistical evaluations?** | Yes |
| **Remarks for Results** | Hepatomas were observed as early as 11 months. At 14 months, 73% of the males (3.5 hepatomas/mouse) and 24% of control males (0.6 hepatomas/mouse) exhibited hepatomas. The incidence of hepatomas in females (9%, 0.1 hepatomas/mouse) was not statistically different from control females (2%, 0.02 hepatomas/mouse) [Miller *et al*., 1983] |
| **Data Qualities Reliabilities** | Reliability code 2. Reliable with restriction. |
| **Remarks for Data Reliability** | Code 2. Basic data given: comparable to guidelines/standards. |
| **References** | Miller, E.C., A.B. Swanson, D.H. Phillips, T.L. Fletcher, A. Liem, and J.A. Miller. (1983) Structure-activity studies of the carcinogenicities in the mouse and rat of some naturally occurring and synthetic alkenylbenzene derivatives related to safrole and estragole. Cancer Research, 43, 1124-1134. |

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| **Substance Name** | Estragole |
| **CAS No.** | 140-67-0 |
| **Remarks for Substance** | The metabolites, 1-hydroxyestragole and estragole epoxide, were also evaluated. |
| **Method/guideline** | Carcinogenesis study |
| **GLP** | Ambiguous |
| **Year** | 1983 |
| **Species/strain** | Mice/CD-1 |
| **Sex** | Male and Female |
| **Route of Administration** | Intraperitoneal |
| **Doses/concentration Levels** | 9.45 mmol/mouse of estragole or estragole epoxide or 1.87 mmoles/mouse of 1'-hydroxyestragole by intraperitoneal injection distributed in a ratio of 1:2:4:8 on days 1, 8, 15, and 22, respectively, of life. These doses correspond to 0.63, 1.26, 2.52, and 5.04 mmol/mouse, respectively. |
| **Exposure Period** | 22 days |
| **Frequency of Treatment** | Days 1, 8, 15, and 22 of life |
| **Control Group** | Yes |
| **Post Exposure** | 13 months |
| **Remarks for Test Conditions** | Male (50) and female (50) CD-1 mice were administered a total dose of 9.45 mmol/mouse of estragole or estragole epoxide or 1.87 mmoles/mouse of 1'-hydroxyestragole by intraperitoneal |

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| | injection distributed in a ratio of 1:2:4:8 on days 1, 8, 15, and 22, respectively, of life. These doses correspond to 0.63, 1.26, 2.52, and 5.04 mmol/mouse, respectively. The mice were weaned at 22 days of age. |
| **Toxic Response/effects by Dose Level** | See remarks for results |
| **Appropriate statistical evaluations?** | Yes |
| **Remarks for Results** | At 12 months, 65% of the mice receiving estragole exhibited hepatomas (1.7 hepatomas/mouse) versus 26% of controls (0.5 hepatomas/mouse) exhibited hepatomas. The incidence of hepatomas in mice given estragole epoxide (40%, 0.6 hepatomas/mouse) was not statistically different from control (26%, 0.5 hepatomas/mouse). For 1'-hydroxyestragole, 93% of the mice receiving the test substance (2.7 hepatomas/mouse) and 15% of control males (0.2 hepatomas/mouse) exhibited hepatomas [Miller *et al*., 1983] |
| **Data Qualities Reliabilities** | Reliability code 2. Reliable with restriction. |
| **Remarks for Data Reliability** | Code 2. Basic data given: comparable to guidelines/standards. |
| **References** | Miller, E.C., A.B. Swanson, D.H. Phillips, T.L. Fletcher, A. Liem, and J.A. Miller. (1983) Structure-activity studies of the carcinogenicities in the mouse and rat of some naturally occurring and synthetic alkenylbenzene derivatives related to safrole and estragole. Cancer Research, 43, 1124-1134. |

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| **Substance Name** | Estragole |
| **CAS No.** | 140-67-0 |
| **Remarks for Substance** | Data is for metabolite, 1-hydroxyestragole |
| **Method/guideline** | Carcinogenesis study |
| **GLP** | Ambiguous |
| **Year** | 1987 |
| **Species/strain** | Mice/Male C57BL/6J x C3H/HeJ F1 |
| **Sex** | Male and Female |
| **Route of Administration** | Intraperitoneal |
| **Doses/concentration Levels** | Dose levels were 0.1 mmol on Day 1, 0.04 mmol on days 8 and 15, and 0.08 mmol on day 22 after birth. The levels are calculated to provide 11.7 on day 1, 18.8 on day 8, 9.3 on day 15 and 10.1 mg/kg bw on day 22, respectively. |
| **Exposure Period** | 22 days |
| **Frequency of Treatment** | Days 1, 8, 15, and 22 of life |

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| **Control Group** | Yes |
| **Post Exposure** | 14 months |
| **Remarks for Test Conditions** | In a study using a hybrid strain of B6C3F1 mice, and the parent strain, C3H/He male and female mice and C57BL/6 male and female mice, the mice were given intraperitoneal injections of 1'-hydroxyestragole on days 1, 8, 15, and 22. Dose levels were 0.1 mmol on Day 1, 0.04 mmol on days 8 and 15, and 0.08 mmol on day 22 after birth. The levels are calculated to provide 11.7 on day 1, 18.8 on day 8, 9.3 on day 15 and 10.1 mg/kg bw on day 22, respectively. The experiment was terminated after 14 months. |
| **Toxic Response/effects by Dose Level** | See remarks for results |
| **Appropriate statistical evaluations?** | Yes |
| **Remarks for Results** | The first tumour-bearing mouse was observed at 10 months. At 12 months, 76% of the treated C3H/He male mice (3.0 hepatomas/mouse) and 26% of control mice (0.3 hepatomas/mouse) exhibited hepatomas. The incidence of hepatomas in C3H/He female mice (6% 0.06 hepatomas/mouse) was not statistically different from those of control females. For C57BL/6 mice, the incidence of hepatomas in treated males was 14% (0.3 hepatomas/mouse) and was 5% (0.07 hepatomas/mouse) in control males. No hepatomas were observed in treated or control B57BL/6 female mice |
| **Data Qualities Reliabilities** | Reliability code 2. Reliable with restriction. |
| **Remarks for Data Reliability** | Code 2. Basic data given: comparable to guidelines/standards. |
| **References** | Wiseman R.W., Miller E.C., Miller J.A. and Liem A. (1987) Structure-activity studies of the hepatocarcinogenicities of alkenylbenzene derivatives related to estragole and safrole on administration to preweanling male C57BL/6J x C3H/HeJ F1 mice. Cancer Research, 47(9), 2275-2283. |

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| **Substance Name** | Estragole |
| **CAS No.** | 140-67-0 |
| **Remarks for Substance** | Data is for metabolite, 1-hydroxyestragole |
| **Method/guideline** | Carcinogenesis study |
| **GLP** | Ambiguous |
| **Year** | 1987 |
| **Species/strain** | Mice/Male B6C3F1 |
| **Sex** | Male |
| **Route of Administration** | Intraperitoneal |

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| **Doses/concentration Levels** | 0.10 mmol/g (15 mg/kg) and 0.01 mmol/g (1.5 mg/kg) |
| **Exposure Period** | Single dose |
| **Frequency of Treatment** | 12 days after birth |
| **Control Group** | Yes |
| **Post Exposure** | 12 months |
| **Remarks for Test Conditions** | Groups of male B6C3F1 mice were given single intraperitoneal injections of 0.10 mmol/g (15 mg/kg) of body weight of 1'-hydroxyestragole 12 days after birth. Animals were sacrificed after 12 months and incidence of hepatic tumors were measured. A second group of males was given a lower dose of 0.01 mmol/g of body weight. |
| **Toxic Response/effects by Dose Level** | See remarks for results |
| **Appropriate statistical evaluations?** | Yes |
| **Remarks for Results** | A statistically significant increase in the incidence of hepatomas/mouse were observed for both substances at 0.1mmol/g bw, but no significant increase was observed at the low dose of 0.01 mmol/g bw (1.5 mg/kg). |
| **Data Qualities Reliabilities** | Reliability code 2. Reliable with restriction. |
| **Remarks for Data Reliability** | Code 2. Basic data given: comparable to guidelines/standards. |
| **References** | Wiseman R.W., Miller E.C., Miller J.A. and Liem A. (1987) Structure-activity studies of the hepatocarcinogenicities of alkenylbenzene derivatives related to estragole and safrole on administration to preweanling male C57BL/6J x C3H/HeJ F1 mice. Cancer Research, 47(9), 2275-2283. |

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| **Substance Name** | Estragole |
| **CAS No.** | 140-67-0 |
| **Remarks for Substance** | The metabolite, 1-hydroxyestragole, was also evaluated. |
| **Method/guideline** | Carcinogenesis study |
| **GLP** | Ambiguous |
| **Year** | 1983 |
| **Species/strain** | Mice/CD-1 |
| **Sex** | Female |
| **Route of Administration** | Oral-Diet |
| **Doses/concentration Levels** | 0, 2300 or 4600 ppm for estragole and 2500 ppm for 1-hydroxyestragole |

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| **Exposure Period** | 12 months |
| **Frequency of Treatment** | Daily |
| **Control Group** | Yes |
| **Remarks for Test Conditions** | In a multipart study evaluating the carcinogenic potential of allylalkoxybenzene derivatives, groups of CD-1 female mice (mean weight 24 g) were maintained on a diet containing 2300 or 4600 ppm estragole or 2500 ppm 1'-hydroxy estragole. The authors estimated that the dietary levels corresponded to an average daily intake of 150-300 and 300-600 mg/kg bw for animals on the 2300 ppm and 4600 ppm estragole diet, respectively, and 180-360 mg/kg bw for animals on the 1'-hydroxyestragole diet. To avoid intolerance the dietary concentration was reduced by 75% for the first 10 days and 50% for the next 10 days. The target diet was then maintained for 12 months. |
| **NOAEL(NOEL)** | Not determined |
| **LOAEL(LOEL)** | 2300 ppm |
| **Actual dose received by dose level and sex** | The authors estimated that the dietary levels corresponded to an average daily intake of 150-300 and 300-600 mg/kg bw for animals on the 2300 ppm and 4600 ppm estragole diet, respectively, and 180-360 mg/kg bw for animals on the 1'-hydroxyestragole diet. |
| **Toxic Response/effects by Dose Level** | See remarks for results |
| **Appropriate statistical evaluations?** | Yes |
| **Remarks for Results** | Survival at 20 months was slightly lower (68-70%) for estragole fed animals compared to control animals (78%). The average life span of mice given 1'-hydroxyestagole was 13.6 months compared to 18 months in controls. Body weights measured at 1, 4, and 8 months were markedly reduced at 4 and 8 months compared to controls. At 10 months, the incidence of hepatomas was 58% for animals at 2300 ppm estragole, 71% for animals at 4600 ppm estragole and 56% for animals at 2500 ppm of 1'-hydroxyestragole and 0 % in controls. Histopathological examinations revealed portal fibrosis, chronic inflammation and bile duct proliferation in addition to the tumours. Varied number of ceroid-laden histocytes and focal area of hyperplasia and megalocytosis were also reported. Four mice fed 4600 ppm estragole had hepatic angiosarcomas |
| **Data Qualities Reliabilities** | Reliability code 2. Reliable with restriction. |
| **Remarks for Data Reliability** | Code 2. Basic data given: comparable to guidelines/standards. |
| **References** | Miller, E.C., A.B. Swanson, D.H. Phillips, T.L. Fletcher, A. Liem, and J.A. Miller. (1983) Structure-activity studies of the carcinogenicities in the mouse and rat of some naturally occurring and synthetic alkenylbenzene derivatives related to safrole and estragole. Cancer Research 43, 1124-1134. |

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| **Substance Name** | Methyl eugenol (surrogate for estragole) |
| **CAS No.** | 93-15-2 |
| **Remarks for Substance** | Data is for structurally related alkoxybenzene derivative, methyl eugenol. Purity greater than 99% |
| **Method/guideline** | National Toxicology Program. Toxicology and Carcinogenesis study NTP TR 491 |
| **GLP** | Yes |
| **Year** | 1998 |
| **Species/strain** | Rat/F344/N |
| **Sex** | Male and Female |
| **Route of Administration** | Oral-Gavage |
| **Doses/concentration Levels** | 0, 37, 75, or 150 mg/kg bw/d; stop exposure group 300 mg/kg bw/d |
| **Exposure Period** | 105 weeks |
| **Frequency of Treatment** | Daily (5 days/week) |
| **Control Group** | Yes |
| **Post Exposure** | 52 weeks for the stop exposure group |
| **Remarks for Test Conditions** | Groups of fifty male and fifty female rats each were administered 0, 37, 75 or 150 mg/kg bw/d methyl eugenol in 0.5% methyl cellulose via gavage once per day, five days a week for 105 weeks. Animals were housed five per cage and fed ad libitum. The animals were observed twice per day and weighed once per week for 12 weeks and once per month thereafter. Necropsies were performed on all animals. Histological examinations were performed on all animals dying during the study; all vehicle control; all low dose female rats and all high dose animals. Tissues examined included adrenal glands, brain, cecum, colon, costochondral junction, duodenum, epididymus/seminal vesicles/tunica vaginalis/scrotal sac/prostrate/testes or ovaries/uterus, esophagus, eyes, femur or sternebrae or vertebrae including marrow, gross lesions and tissue masses with regional lymph nodes, heart, ileum, jejunum, kidneys, larynx and pharynx, liver, lungs and bronchi, mammary gland, mandibular or mesenteric lymph nodes, nasal cavity and turbinates, oral cavity, pancreas, parathyroids, pituitary gland, preputial or clitoral gland, rectum, salivary glands, sciatic nerve, skin, spinal cord, spleen, stomach, thigh muscle, thymus, thyroid gland, trachea, urinary bladder and Zymbal gland. Tissues examined in low dose male rat groups included adrenal glands, kidney, liver, spleen, and testis. |
| **NOAEL(NOEL)** | Not determined |

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| **LOAEL(LOEL)** | 37 mg/kg bw/d |
| **Toxic Response/effects by Dose Level** | See remarks for results. |
| **Appropriate statistical evaluations?** | Yes |
| **Remarks for results** | All 150 and 300 mg/kg males died before the end of the study. Mean body weights of all dosed groups were less than those of the vehicle controls throughout the study. The incidences of liver non-neoplastic lesions in dosed groups of male and females were increased at 6 months, 12 months, and 2 years. There were statistically significant increases in oval cell hyperplasia, hepatocyte hypertrophy, and eosinophilic foci, at all dose levels in male and female rats. At the three highest doses (75, 150, and 300 mg/kg bw per day) atypical focal bile duct hyperplasia, focal cystic degeneration, and mixed cell foci were observed, more in males than females. Many of the same non-neoplastic lesions of the liver were reported in the 300 mg/kg bw groups of male and female rats at both 6 and 12 months in the stop-exposure group. Non-neoplastic lesions of the glandular stomach included statistically significant increases in mucosal atrophy at all dose levels and neuroendocrine hyperplasia at the three highest dose levels in females and at all dose levels in males. There was a significant increase in the incidence of nephropathy in females at 300 mg/kg, and the incidence of renal tubule hyperplasia was greater in the greater than 75 mg/kg groups than in the vehicle control.<br><br>Methyl eugenol-related liver neoplasms occurred in all dosed groups and comprised hepatocellular adenomas and carcinomas, hepatocholangiomas, and hepatocholangiocarcinomas. There was a statistically significant increase (P equals 0.049 in males and P equals 0.017 in females at 37 mg/kg bw; P less than 0.001 for all other treated groups) in the incidence of hepatocellular adenomas and carcinomas in all dose groups of males and female rats. Hepatocholangiomas and hepatocholangiocarcinomas were reported in the 150 mg/kg bw group of males (2/50, 4%) and females (3/49, 6%) and at higher incidence in the 300 mg/kg bw stop-exposure groups of males (13/50, 26%) and females (17/50, 34%). The appearance of cholangiocarcinomas and bile duct dysplasia was said to provide some additional evidence of carcinogenicity based on the rarity of these lesions in F344/N rats (historical incidence, 3/2145, 0.1%).<br><br>Both benign (3/50, 6%) and malignant (4/50, 8%) neuroendocrine cell neoplasms of the glandular stomach were reported in males at 150 mg/kg bw and in the 300 mg/kg bw stop-exposure group (2/49, 4.1% benign and 2/49, 4.1% malignant). The incidence of these neoplasms was much higher in females at dose levels of 75 mg/kg bw (13/50, 26% benign and 12/50, 24% malignant) and greater. |

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| | There were also significant increases in the incidence of: malignant mesothelioma in male rats given greater than 150 mg/kg; and of mammary gland fibroadenoma in 75 and 150 mg/kg males; and fibroma of the subcutaneous tissue in 37 and 75 mg/kg males. These neoplasms were not found in female rats at any dose level. |
| **Conclusion Remarks** | The authors determined that under the conditions of these 2-year gavage studies there was clear evidence of carcinogenic activity of methyl eugenol as shown by increased incidences of liver neoplasms and neuroendocrine tumors of the glandular stomach in male and female rats and the increased incidences of kidney neoplasms, malignant mesothelioma, mammary gland fibroadenoma, and subcutaneous fibroma and fibroma or fibrosarcoma in male rats. However, because of the evidence of toxicity of methyl eugenol in all groups of rats and mice, the study cannot be recognized as conclusive for carcinogenicity at lower, non-toxic doses. In particular, the hepatic damage undoubtedly altered the metabolism of the compound, and the gastric damage probably altered its absorption. |
| **Data Qualities Reliabilities** | Reliability code 1. Reliable without restriction. |
| **Remarks for Data Reliability** | Code 1. Guideline study. |
| **References** | National Toxicology Program (NTP) (2000) Toxicology and carcinogenesis studies of methyl eugenol in F344/N Rats and B6C3F1 mice. NTP-TR-491. U.S. Dept of Health and Human Services. NIH Publication No. 98-3950. |

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| **Substance Name** | Methyl eugenol (surrogate for estragole) |
| **CAS No.** | 93-15-2 |
| **Remarks for Substance** | Data is for structurally related alkoxybenzene derivative, methyl eugenol. Purity greater than 99% |
| **Method/guideline** | National Toxicology Program. Toxicology and Carcinogenesis study NTP TR 347 |
| **GLP** | Yes |
| **Year** | 1998 |
| **Species/strain** | Mice/B6C3F1 |
| **Sex** | Male and Female |
| **Route of Administration** | Oral-Gavage |
| **Doses/concentration Levels** | 0, 37, 75, or 150 mg/kg bw/d |
| **Exposure Period** | 104 weeks |
| **Frequency of Treatment** | Daily (5 days/week) |
| **Control Group** | Yes |

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| **Remarks for Test Conditions** | Groups of fifty male and fifty female mice each were administered 0, 37, 75 or 150 mg/kg bw/d methyl eugenol in 0.5% methyl cellulose via gavage once per day, five days a week for 104 weeks. Animals were housed five per cage and fed ad libitum. The animals were observed twice per day and weighed once per week for 12 weeks and once per month thereafter. Necropsies were performed on all animals. Histological examinations were performed on all animals dying during the study, all vehicle controls, and all high dose animals. Tissues examined included adrenal glands, brain, cecum, colon, costochondral junction, duodenum, epididymus/seminal vesicles/tunica vaginalis/scrotal sac/prostrate/testes or ovaries/uterus, esophagus, eyes, femur or sternebrae or vertebrae including marrow, gallbladder, gross lesions and tissue masses with regional lymph nodes, heart, ileum, jejunum, kidneys, larynx and pharynx, liver, lungs and bronchi, mammary gland, mandibular or mesenteric lymph nodes, nasal cavity and turbinates, oral cavity, pancreas, parathyroids, pituitary gland, preputial or clitoral gland, rectum, salivary glands, sciatic nerve, skin, spinal cord, spleen, stomach, thigh muscle, thymus, thyroid gland, trachea, urinary bladder and Zymbal gland. |
| **NOAEL(NOEL)** | Not determined |
| **LOAEL(LOEL)** | 37 mg/kg bw/d (females) |
| **Toxic Response/effects by Dose Level** | See remarks for results |
| **Appropriate statistical evaluations?** | Yes |
| **Remarks for Results** | Survival of all dosed groups of male mice was similar to that of the vehicle controls. The survival of treated females was significantly less than those reported for control animals. Mean body weights of dosed mice were reported to be "generally less than those of the vehicle controls throughout the studies". In female mice and, to a lesser extent, in male mice there was evidence of hepatotoxicity of methyl eugenol. Significant increases in oval cell hyperplasia, eosinophilic foci, hepatocyte hypertrophy and necrosis, haematopoietic cell proliferation, haemosiderin pigmentation, and bile duct cysts were observed at all dose levels in male and female mice. Non-neoplastic lesions of the glandular stomach included statistically significant increases in hyperplasia, ectasia, atrophy at all dose levels in both males and females and mineralization and necrosis in lower incidence also in both sexes incidences of chronic atrophic gastritis was high. Gastric tumours were found in two high dose males. The incidence of hepatocellular adenomas, hepatocellular carcinomas and hepatoblastomas was high in both treated and control male and female mice. While control males and females showed tumour rates of 63% (31/49) and 50% (25/50), respectively, and all treatment groups of males and females had tumour rates in excess of 92% with the exception of high dose male rates in which the tumour rate was 82% (41/50). Evidence of infection by *H. hepaticus* was found by PCR-RFLP, but associated hepatitis was not found. |

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| **Conclusion Remarks** | The authors determined that under the conditions of these 2-year gavage studies there was evidence of carcinogenic activity of methyl eugenol for male or female B6C3F1 mice at the dose levels tested. |
| **Data Qualities Reliabilities** | Reliability code 1. Reliable without restriction. |
| **Remarks for Data Reliability** | Code 1. Guideline study. |
| **References** | National Toxicology Program (NTP) (2000) Toxicology and carcinogenesis studies of methyl eugenol in F344/N Rats and B6C3F1 mice. NTP-TR-491. U.S. Dept of Health and Human Services. NIH Publication No. 98-3950. |

## 4.4 Reproductive Toxicity

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| **Substance Name** | Estragole |
| **CAS No.** | 140-67-0 |
| **Remarks for Substance** | Data is for oil of nutmeg containing 10-20% p-allylalkoxybenzene derivatives, myristicin, elemicin, safrole, and methyl eugenol. Members of this class of substance including estragole are known to metabolize via a 1'-hydroxylation pathway to yield a reactive hepatotoxic metabolite. |
| **Test Type** | One generation |
| **GLP** | No |
| **Year** | 1973 |
| **Species/Strain** | Mouse/CD-1 outbred |
| **Sex** | Female |
| **Route of Administration** | Oral-Gavage |
| **Duration of Test** | Days 6 to 15 of gestation |
| **Doses/Concentration** | 0(control), 6, 26, 120, 560 mg/kg bw/day and a positive control of 150 mg/kg bw/day of aspirin. |
| **Premating Exposure period for males** | None |
| **Premating Exposure period for females** | None |
| **Frequency of Treatment** | Daily |
| **Control Group and Treatment** | Control group received corn oil vehicle (10 ml//kg); Positive control received 150 mg/kg bw/day of aspirin in corn oil. |
| **Remarks for Test Conditions** | Study measured parameters for reproductive and developmental toxicity. In the reproductive segment of the study, virgin adult female CD-1 outbred mice were gang- |

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| | housed in plastic disposable cages in a temperature- and humidity-controlled room. Animals were given free access to food and fresh tap water. There were mated with untreated young adult males and observation of vaginal sperm plugs was considered day 0 of gestation. Beginning on Day 6 and continuing daily through Day 15 of gestation, females were given 0, 6, 26, 120, or 560 mg/kg bw of the test material (FDA 71-28) by gavage in corn oil. A positive control group received 150 mg/kg bw/day of aspirin. Body weights were recorded on days 0, 6, 11, 15, and 17 of gestation. Females were observed daily for appearance and behavior. Food consumption and body weight were monitored to eliminate any abnormalities that may be associated with anorexia in pregnant females. On Day 17 all dams were subjected to Caesarian section and the number of implantation sites, resorption sites, live fetuses, dead fetuses, and body weight of live pups were recorded. Gestation index, mortality, gross pathology incidence of the dam urogenital tract, number of implantation sites, number of corpora lutea, litter size and weights, sex and sex ratio of pups, and gross abnormalities to pups were reported. The urogenital tract of each dam was examined for anatomical abnormalities. One-third of fetuses of each litter underwent detailed visceral examination at 10x magnification. The remaining two-thirds were stained with alizarin red S dye/KOH and examined for skeletal defects. |
| **NOAEL(NOEL)** | >560 mg/kg bw/day (equivalent to approximately 112 mg/kg/d of allylalkoxybenzene derivatives) |
| **Actual dose received by dose level and sex** | >560 mg/kg bw/day |
| **Parental data and F1 as appropriate** | Data for number of females mated/pregnant at each dose level: 0 mg/kg bw, 24/21; 150 mg/kg bw of aspirin, 30/20; 6 mg/kg bw, 30/22; 26 mg/kg bw, 31/21;120 mg/kg bw, 22/21; 560 mg/kg bw, 32/20. All pregnant females survived to sacrifice on Day 17. There was no significant difference in dam body weights between controls and any test group measured at Days 0, 6, 11, 15, or 17 of the study. None of the pregnant females died or aborted before Day 17and all litters were alive on Day 17 sacrifice. Average number of corpora lutea/dam mated were similar for controls and treatment groups: 0 mg/kg bw, 12.5; 150 mg/kg bw aspirin, 12.0; 6 mg/kg bw, 12.3; 26 mg/kg bw, 11.2; 120 mg/kg bw, 12.9; 560 mg/kg bw, 11.2. The average number of implantation sites/dam and % partial resorptions were similar for all groups:0 mg/kg bw, 11.8 and 19%; 150 mg/kg bw aspirin, 11.3 and 45%; 6 mg/kg bw, 12.5 and 45%; 26 mg/kg bw, 11.9 and 28%; 120 mg/kg bw, 10.5 and 28%; 560 mg/kg bw, 11.0 and 25%. Based on bodyweight changes, clinical observation, and gross examination of the urogenital tract, was no evidence of toxicity to dams. |
| **Offspring toxicity F1 and F2** | Based on gross examination of live pups, visceral examination and skeletal examination there were no signs of toxicity to offspring. The total number of live fetuses, average number of live fetuses per dam, sex ratio, number of dead fetuses, and average fetal weight were not different between control and treatment groups. Total number of live fetuses/dead |

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| **Conclusion remarks** | The administration of up to and including 560 mg/kg bw/day of test article FDA 71-28 to pregnant mice on days 6 through 15 of gestation had no effects on nidation, maternal survival or fetal survival. The number and types of abnormalities seen in tissues of the dam or pups of the test groups did not differ for the number and type occurring spontaneously in the positive or negative controls. |
| **Data Reliabilities Qualities** | Reliability code 2. Reliable with restriction. |
| **Remarks for Data Reliability** | Code 2. Acceptable, well-documented publication/study report, which meets basic scientific principles. |
| **References** | Morgareidge K. (1973a) Teratologic evaluation of FDA 71-28 in mice. Contract No. FDA 71-260. Unpublished report. |

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| **Substance Name** | Estragole |
| **CAS No.** | 140-67-0 |
| **Remarks for Substance** | Data is for oil of nutmeg containing 10-20% p-allylalkoxybenzene derivatives, myristicin, elemicin, safrole, and methyl eugenol |
| **Test Type** | One generation |
| **GLP** | No |
| **Year** | 1973 |
| **Species/Strain** | Hamster/adult golden |
| **Sex** | Female |
| **Route of Administration** | Oral-Gavage |
| **Duration of Test** | Days 6 to 10 of gestation |
| **Doses/Concentration** | 0(control), 6, 28, 130, or 600 mg/kg bw/day and a positive control of 250 mg/kg bw/day of aspirin |
| **Premating Exposure period for males** | None |
| **Premating Exposure period for females** | None |
| **Frequency of Treatment** | Daily |
| **Control Group and Treatment** | Control group received corn oil vehicle (10 ml//kg); Positive control received 250 mg/kg bw/day of aspirin in corn oil. |
| **Remarks for Test Conditions** | Study measured parameters for reproductive and developmental toxicity. In the reproductive segment of the study, groups (26-28/dose/group) of virgin adult female hamster were individually housed in mess-bottom cages in a temperature- and humidity-controlled room. Animals were given free access to food and fresh tap water. There were mated one to one with untreated adult males and the appearance of motile sperm in the vaginal sperm was considered day 0 of gestation. |

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| | Beginning on Day 6 and continuing daily through Day 10 of gestation, females were given 0, 6, 28, 130, or 600 mg/kg bw of the test material (FDA 71-28) by gavage in corn oil. A positive control group received 250 mg/kg bw/day of aspirin. Body weights were recorded on days 0, 8, 10, and 14 of gestation. Females were observed daily for appearance and behavior. Food consumption and body weight were monitored to eliminate any abnormalities that may be associated with anorexia in pregnant females. On Day 14 all dams were subjected to Caesarian section and the number of implantation sites, resorption sites, live fetuses, dead fetuses, and body weight of live pups were recorded. Gestation index, mortality, gross pathology incidence of the dam urogenital tract, number of implantation sites, number of corpora lutea, litter size and weights, sex and sex ratio of pups, and gross abnormalities to pups were reported. The urogenital tract of each dam was examined for anatomical abnormalities. One-third of fetuses of each litter underwent detailed visceral examination at 10x magnification. The remaining two-thirds were stained with alizarin red S dye/KOH and examined for skeletal defects. |
| **NOAEL(NOEL)** | >600 mg/kg bw/day (equivalent to approximately 120 mg/kg/d of allylalkoxybenzene derivatives) |
| **Actual dose received by dose level and sex** | >600 mg/kg bw/day |
| **Parental data and F1 as appropriate** | Data for number of females mated/ pregnant at each dose level: 0 mg/kg bw, 27/21; 250 mg/kg bw of aspirin, 26/19; 6 mg/kg bw, 28/19; 28 mg/kg bw, 26/21; 130 mg/kg bw, 28/20; 600 mg/kg bw, 27/23. All pregnant females survived to sacrifice on Day 14. There was no significant difference in dam body weights between controls and any test group measured at Days 0, 6, 8, 10, or 14 of the study. One death each was reported in the two control groups and in the two highest dose groups before day 14. All litters were alive on Day 14 sacrifice. Average number of corpora lutea/dam mated were similar for controls and treatment groups: 0 mg/kg bw, 10.3; 250 mg/kg bw aspirin, 9.9; 6 mg/kg bw, 9.6; 28 mg/kg bw, 11.4; 130 mg/kg bw, 9.6; 600 mg/kg bw, 11.2. The average number of implantation sites/dam and % partial resorptions were similar for all groups:0 mg/kg bw, 11.7 and 15%; 250 mg/kg bw aspirin, 11.3 and 39%; 6 mg/kg bw, 12.1 and 32%; 28 mg/kg bw, 11.9 and 38%; 130 mg/kg bw, 11.5 and 42%; 600 mg/kg bw, 12.1 and 23%. Based on bodyweight changes, clinical observation, and gross examination of the urogenital tract, was no evidence of toxicity to dams. |
| **Offspring toxicity F1 and F2** | Based on gross examination of live pups, visceral examination, and skeletal examination there were no signs of toxicity to offspring in either the control or test groups. The total number of live fetuses, average number of live fetuses per dam, sex ratio, and average fetal weight were not different between control and treatment groups. A small number of dead fetuses |
| **Conclusion remarks** | The administration of up to and including 600 mg/kg bw/day of test article FDA 71-28 to pregnant golden hamsters on days 6 through 10 of gestation had no effects on nidation, maternal survival or fetal survival. The number and types of |

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| | abnormalities seen in tissues of the dam or pups of the test groups did not differ for the number and type occurring spontaneously in the positive or negative controls. |
| **Data Reliabilities Qualities** | Reliability code 2. Reliable with restriction. |
| **Remarks for Data Reliability** | Code 2. Acceptable, well-documented publication/study report, which meets basic scientific principles. |
| **References** | Morgareidge K. (1973b) Teratologic evaluation of FDA 71-28 in hamsters. Contract No. FDA 71-260. Unpublished report. |

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| **Substance Name** | Estragole |
| **CAS No.** | 140-67-0 |
| **Test Type** | One generation |
| **GLP** | No |
| **Year** | 1973 |
| **Species/Strain** | Rat/adult Wistar |
| **Sex** | Female |
| **Route of Administration** | Oral-Gavage |
| **Duration of Test** | Days 6 to 14 of gestation |
| **Doses/Concentration** | 0(control), 3, 12, 56, or 260 mg/kg bw/day and a positive control of 250 mg/kg bw/day of aspirin. |
| **Premating Exposure period for males** | None |
| **Premating Exposure period for females** | None |
| **Frequency of Treatment** | Daily |
| **Control Group and Treatment** | Control group received corn oil vehicle (10 ml//kg); Positive control received 250 mg/kg bw/day of aspirin in corn oil. |
| **Remarks for Test Conditions** | Study measured parameters for reproductive and developmental toxicity. In the reproductive segment of the study, virgin adult female Wistar were individually housed in mess-bottom cages in a temperature- and humidity-controlled room. Animals were given free access to food and fresh tap water. There were mated with untreated young adult males and observation of vaginal sperm plugs was considered day 0 of gestation. Beginning on Day 6 and continuing daily through Day 15 of gestation, females were given 0, 3, 2, 56, or 260 mg/kg bw of the test material (FDA 71-28) by gavage in corn oil. A positive control group received 250 mg/kg bw/day of aspirin. Body weights were recorded on days 0, 6, 11, 15, and 20 of gestation. Females were observed daily for appearance and behavior. Food consumption and body weight were monitored to eliminate any abnormalities that may be associated with anorexia in pregnant females. On Day 20 all dams were |

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| | subjected to Caesarian section and the number of implantation sites, resorption sites, live fetuses, dead fetuses, and body weight of live pups were recorded. Gestation index, mortality, gross pathology incidence of the dam urogenital tract, number of implantation sites, number of corpora lutea, litter size and weights, sex and sex ratio of pups, and gross abnormalities to pups were reported. The urogenital tract of each dam was examined for anatomical abnormalities. One-third of fetuses of each litter underwent detailed visceral examination at 10x magnification. The remaining two-thirds were stained with alizarin red S dye/KOH and examined for skeletal defects. |
| **NOAEL(NOEL)** | >260 mg/kg bw/day (equivalent to approximately 52 mg/kg/d of allylalkoxybenzene derivatives) |
| **Actual dose received by dose level and sex** | >260 mg/kg bw/day |
| **Parental data and F1 as appropriate** | Data for number of females mated/ pregnant at each dose level: 0 mg/kg bw, 25/23; 250 mg/kg bw of aspirin, 25/22; 3 mg/kg bw, 25/25; 12 mg/kg bw, 25/23; 56 mg/kg bw, 25/22; 260 mg/kg bw, 25/21. All pregnant females survived to sacrifice on Day 20. There was no significant difference in dam body weights between controls and any test group measured at Days 0, 6, 11, 15, or 20 of the study. None of the pregnant females died or aborted before Day 20 and all litters were alive on Day 20 sacrifice. Average number of corpora lutea/dam mated were similar for controls and treatment groups: 0 mg/kg bw, 12.8; 250 mg/kg bw aspirin, 11.1; 3 mg/kg bw, 12.7; 12 mg/kg bw, 12.5; 56 mg/kg bw, 11.6; 260 mg/kg bw, 10.7. The average number of implantation sites/dam and % partial resorptions were similar for all groups:0 mg/kg bw, 11.9 and 9%; 250 mg/kg bw aspirin, 11.1 and 32%; 3 mg/kg bw, 12 and 12%; 12 mg/kg bw, 11.8 and 4%; 56 mg/kg bw, 11.1 and 5%; 260 mg/kg bw, 11.1 and 5%. Based on bodyweight changes, clinical observation, and gross examination of the urogenital tract, there was no evidence of toxicity to dams. |
| **Offspring toxicity F1 and F2** | Based on gross examination of live pups, visceral examination, and skeletal examination there were no signs of toxicity to offspring in either the control or test groups. The total number of live fetuses, average number of live fetuses per dam, sex ratio, and average fetal weight were not different between control and treatment groups. A small number of dead fetuses |
| **Conclusion Remarks** | The administration of up to and including 260 mg/kg bw/day of test article FDA 71-28 to pregnant Wistar rats on days 6 through 15 of gestation had no effects on nidation, maternal survival or fetal survival. The number and types of abnormalities seen in tissues of the dam or pups of the test groups did not differ for the number and type occurring spontaneously in the positive or negative controls. |
| **Data Reliabilities Qualities** | Reliability code 2. Reliable with restriction. |
| **Remarks for Data Reliability** | Code 2. Acceptable, well-documented publication/study report, which meets basic scientific principles. |
| **References** | Morgareidge K. (1973c) Teratologic evaluation of FDA 71-28 in rats. Contract No. FDA 71-260. Unpublished report. |

## 4.5 Developmental/Teratogenicity Toxicity

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| **Substance Name** | Safrole (analog for estragole) |
| **CAS No.** | 94-59-7 |
| **Remarks for Substance** | Data is for surrogate chemical 3,4-dimethyleneoxyallylbenzene (safrole). Substance is known to form reactive 1'-hydroxy metabolite (Miller et al., 1983) |
| **Test Type** | Developmental toxicity |
| **GLP** | No |
| **Year** | 1985 |
| **Species/strain** | Mice/Swiss |
| **Sex** | Female |
| **Route of Administration** | Oral-Gavage |
| **Duration of Test** | Days 6-14 of gestation |
| **Doses/concentration Levels** | 0, 5, 50, 100, 150, or 200 mg/kg bw/day |
| **Exposure Period** | 8 days |
| **Frequency of Treatment** | Daily |
| **Control Group and Treatment** | Olive oil vehicle |
| **Remarks for Test Conditions** | Groups of 15-25 female Swiss mice were given oral doses of safrole by gavage daily at 0, 5, 50, 100, 150, or 200 mg/kg bw/day in olive oil for 8 days from days 6 to 14 of gestation. Males were untreated. Pregnant females were sacrificed on day 18. Parameters monitored included survival of females, number pregnant on day 18, number of implantations, number and % reabsorbed, number of live foetuses, mean foetal weight, and number and % of malformations per dosed group. Malformations were further classified according to anomalies of the cranium, anterior and posterior phalangi, column vertebrate, and morphological irregularities and absence of sternbrae in untreated and treated groups. Foetal malformations of the palate, brain, limbs, and tail were also recorded. |
| **NOAEL(NOEL) maternal toxicity** | 5 mg/kg bw/day |
| **LOAEL(LOEL) maternal toxicity** | 50 mg/kg bw/day |
| **NOAEL (NOEL)** | 5 mg/kg bw/day based on growth retardation in fetuses at |

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| **developmental toxicity** | higher dose levels |
| **LOAEL (LOEL) developmental toxicity** | 50 mg/kg/day |
| **Actual dose received by dose level and sex** | 0, 5, 50, 100, 150, or 200 mg/kg bw/day |
| **Maternal data with dose level** | Maternal data indicated that survival among females was decreased in a dose dependent manner at dose levels of 100 mg/kg bw/day and above. The number pregnant was decreased at 100 (, 150 and 200 mg/kg bw/day but this paralleled survival rates. The number of implantations also decreased at dose levels of 100 mg/kg bw/day and above. Signs of maternal toxicity were recorded at dose levels equal to and equal to and greater than 50 mg/kg bw/day. The % reabsorptions increased at doses equal to and greater than 50 mg/kg bw/day. |
| **Fetal Data with Dose Level** | The mean foetal weight decreased at 50 mg/kg bw/day and above. There was no significant difference between foetal weight and survival between the controls and the 5 mg/kg/day dosed group. Although there was a statistically significant ($p<0.001$) in malformations at the 50 and 150 mg/kg bw/day dosed groups there was no dose response. The authors noted that although there were significant signs of toxicity to dams and foetuses, there was no significant increase in malformations in the treated groups when compared to the control group. % malformations: control 9.2%, 5 mg/kg bw/day, 13.2%; 50 mg/kg bw/day, 19.4%; 100 mg/kg bw/day, 15.2%; 150 mg/kg bw/day, 19.4%<br><br>The only consistent anomalies observed in treated groups were a malformation to the anterior and posterior phalange, but there was no direct dose dependent change among treated groups. |
| **Appropriate statistical evaluations** | Not given |
| **Conclusion Results** | Safrole did not cause any significant increase in malformations in mice foetuses at all administered dose levels. Maternal toxicity and foetal toxicity were noted at doses equal to and greater than 50 mg/kg bw/day. |
| **Data Qualities Reliabilities** | Reliability code 123. Reliable with restrictions. |
| **Remarks for Data Reliability** | Code 2. Comparable to guideline study. |
| **References** | Moro M.G., Ognio E., Rossi L, Ferreri Santi L., and Santi L. (1985) Prenatal toxicity of safrole in laboratory animals. Rivista Tossicologia Sperimentale Clinica, 15(1-2), 91-97. |

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| **Substance Name** | Estragole |
| **CAS No.** | 140-67-0 |
| **Remarks for Substance** | Data is for oil of nutmeg containing 10-20% p-allylalkoxybenzene derivatives, myristicin, elemicin, safrole, and |

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| | methyl eugenol Note: all substance mentioned arer metabolized via 1'hydroxylation. |
| **Test Type** | Teratology study |
| **GLP** | No |
| **Year** | 1973 |
| **Species/strain** | Mouse/CD-1 outbred |
| **Sex** | Female |
| **Route of Administration** | Oral-Gavage |
| **Duration of Test** | 10 days |
| **Doses/concentration Levels** | 0(control), 6, 26, 120, 560 mg/kg bw/day and a positive control of 150 mg/kg bw/day of aspirin |
| **Exposure Period** | Days 6 to 15 of gestation |
| **Frequency of Treatment** | Daily |
| **Control Group and Treatment** | Control group received corn oil vehicle (10 ml//kg); Positive control received 150 mg/kg bw/day of aspirin in corn oil |
| **Remarks for Test Conditions** | Study measured parameters for reproductive and developmental toxicity. In the study, virgin adult female CD-1 outbred mice were gang-housed in plastic disposable cages in a temperature- and humidity-controlled room. Animals were given free access to food and fresh tap water. There were mated with untreated young adult males and observation of vaginal sperm plugs was considered day 0 of gestation. Beginning on Day 6 and continuing daily through Day 15 of gestation, groups (20-22/group) of pregnant females were given 0, 6, 26, 120, or 560 mg/kg bw of the test material (FDA 71-28) by gavage in corn oil. A positive control group received 150 mg/kg bw/day of aspirin. Body weights were recorded on days 0, 6, 11, 15, and 17 of gestation. Females were observed daily for appearance and behavior. Food consumption and body weight were monitored to eliminate any abnormalities that may be associated with anorexia in pregnant females. On Day 17 all dams were subjected to Caesarian section and the number of implantation sites, resorption sites, live fetuses, dead fetuses, and body weight of live pups were recorded. Gestation index, mortality, gross pathology incidence of the dam urogenital tract, number of implantation sites, number of corpora lutea, litter size and weights, sex and sex ratio of pups, and gross abnormalities to pups were reported (these data were described in the robust summary for reproductive effects for the test material). The urogenital tract of each dam was examined for anatomical abnormalities. One-third of fetuses of each litter underwent detailed visceral examination at 10x magnification. The remaining two-thirds were stained with alizarin red S dye/KOH and examined for skeletal defects (the maternal and developmental fetal effects are discussed in this robust summary). |

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| **NOAEL(NOEL) maternal toxicity** | >560 mg/kg bw/day (approximately equal to a daily dose of 112 mg/kg bw for allylalkoxybenzene derivatives) |
| **NOAEL (NOEL) developmental toxicity** | >560 mg/kg bw/day |
| **Actual dose received by dose level and sex** | 0, 6, 26, 120, or 560 mg/kg bw of the test material (FDA 71-28) |
| **Maternal data with dose level** | Daily clinical observation and measurement of body weight gain failed to show any differences between control and test groups of female mice. The number pregnant and % pregnancy were similar for all dose and control groups. No abortions were observed in any group. |
| **Fetal Data with Dose Level** | The average fetal weight of treatment and control groups were not statistically different ($p>0.05$). The total number of live fetuses were similar for test and control groups. Also, there was no significant difference in the number of dead fetuses between test and control groups. Skeletal examination of sternebrae showed no significant differences in the incidence of incomplete ossification or missing sternebrae for test and control groups. Likewise the incidences of fetuses with more than 13 ribs, incomplete ossification of vertebrae and extremities, incomplete skull closure was similar for test and control animals. Visceral examination failed to reveal any evidence of abnormalities at any dose level. |
| **Conclusion Results** | There was no evidence of maternal toxicity or developmental toxicity at dose levels up to and including 560 mg/kg bw/day of test material. |
| **Data Qualities Reliabilities** | Reliability code 2. Reliable with restriction. |
| **Remarks for Data Reliability** | Code 2. Acceptable, well-documented publication/study report, which meets basic scientific principles. |
| **References** | Morgareidge K. (1973a) Teratologic evaluation of FDA 71-28 in mice. Contract No. FDA 71-260. Unpublished report. |

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| **Substance Name** | Estragole |
| **CAS No.** | 140-67-0 |
| **Remarks for Substance** | Data is for oil of nutmeg containing 10-20% p-allylalkoxybenzene derivatives, myristicin, elemicin, safrole, and methyl eugenol |
| **Test Type** | Teratology study |
| **GLP** | No |
| **Year** | 1973 |
| **Species/strain** | Rat/female Wistar |
| **Sex** | Female |
| **Route of Administration** | Oral-Gavage |

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| **Duration of Test** | 10 days |
| **Doses/concentration Levels** | 0(control), 3, 12, 56, 260 mg/kg bw/day and a positive control of 250 mg/kg bw/day of aspirin |
| **Exposure Period** | Days 6 to 15 of gestation |
| **Frequency of Treatment** | Daily |
| **Control Group and Treatment** | Control group received corn oil vehicle (10 ml//kg); Positive control received 250 mg/kg bw/day of aspirin in corn oil |
| **Remarks for Test Conditions** | Study measured parameters for reproductive and developmental toxicity. In the study, virgin adult female rats were individually housed in mess bottom cages in a temperature- and humidity-controlled room. Animals were given free access to food and fresh tap water. There were mated with untreated young adult males and observation of vaginal sperm plugs was considered day 0 of gestation. Beginning on Day 6 and continuing daily through Day 15 of gestation, groups (21-25/group) of pregnant females were given 0, 6, 26, 120, or 260 mg/kg bw of the test material (FDA 71-28) by gavage in corn oil. A positive control group received 250 mg/kg bw/day of aspirin. Body weights were recorded on days 0, 6, 11, 15, and 20 of gestation. Females were observed daily for appearance and behavior. Food consumption and body weight were monitored to eliminate any abnormalities that may be associated with anorexia in pregnant females. On Day 20 all dams were subjected to Caesarian section and the number of implantation sites, resorption sites, live fetuses, dead fetuses, and body weight of live pups were recorded. Gestation index, mortality, gross pathology incidence of the dam urogenital tract, number of implantation sites, number of corpora lutea, litter size and weights, sex and sex ratio of pups, and gross abnormalities to pups were reported (these data were described in the robust summary for reproductive effects for the test material). The urogenital tract of each dam was examined for anatomical abnormalities. One-third of fetuses of each litter underwent detailed visceral examination at 10x magnification. The remaining two-thirds were stained with alizarin red S dye/KOH and examined for skeletal defects (the maternal and developmental fetal effects are discussed in this robust summary). |
| **NOAEL(NOEL) maternal toxicity** | >260 mg/kg bw/day (approximately equal to a daily dose of 52 mg/kg bw for allylalkoxybenzene derivatives) |
| **NOAEL (NOEL) developmental toxicity** | >260 mg/kg bw/day |
| **Actual dose received by dose level and sex** | 0, 3, 12, 56, or 260 mg/kg bw of the test material (FDA 71-28) |
| **Maternal data with dose level** | Daily clinical observation and measurement of body weight gain failed to show any differences between control and test groups of female rats. The number pregnant and % pregnancy were similar for all dose and control groups. No abortions were observed in any group. |
| **Fetal Data with Dose Level** | The average fetal weight of treatment and control groups were not statistically different ($p>0.05$). The total number of live |

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| | fetuses were similar for test and control groups. Also, there was no significant difference in the number of dead fetuses between test and control groups. Except for positive control group, skeletal examination of sternebrae showed no significant differences in the incidence of incomplete ossification or missing sternebrae for test and untreated control group. Likewise the incidences of fetuses with more than 13 ribs, incomplete ossification of vertebrae and extremities, incomplete skull closure were similar for test and the untreated control group except for the positive aspirin-treated control group in which increases in incidences of these skeletal effects were observed. Visceral examination failed to reveal any evidence of abnormalities at any dose level. |
| **Conclusion Results** | There was no evidence of maternal toxicity or developmental toxicity at dose levels up to and including 260 mg/kg bw/day of test material. |
| **Data Qualities Reliabilities** | Reliability code 2. Reliable with restriction. |
| **Remarks for Data Reliability** | Code 2. Acceptable, well-documented publication/study report, which meets basic scientific principles. |
| **References** | Morgareidge K. (1973c) Teratologic evaluation of FDA 71-28 in rats. Contract No. FDA 71-260. Unpublished report. |

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| **Substance Name** | Estragole |
| **CAS No.** | 140-67-0 |
| **Remarks for Substance** | Data is for oil of nutmeg containing 10-20% p-allylalkoxybenzene derivatives, myristicin, elemicin, safrole, and methyl eugenol |
| **Test Type** | Teratology study |
| **GLP** | No |
| **Year** | 1973 |
| **Species/strain** | Hamster/female golden |
| **Sex** | Female |
| **Route of Administration** | Oral-Gavage |
| **Duration of Test** | 5 days |
| **Doses/concentration Levels** | 0(control), 6, 28, 130, 600 mg/kg bw/day and a positive control of 250 mg/kg bw/day of aspirin |
| **Exposure Period** | Days 6 to 10 of gestation |
| **Frequency of Treatment** | Daily |
| **Control Group and Treatment** | Control group received corn oil vehicle (10 ml//kg); Positive control received 250 mg/kg bw/day of aspirin in corn oil |
| **Remarks for Test Conditions** | Study measured parameters for reproductive and developmental toxicity. In the study, virgin adult female |

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| | hamsters were individually housed in mess bottom cages in a temperature- and humidity-controlled room. Animals were given free access to food and fresh tap water. There were mated one to one with untreated young adult males and the appearance of motile sperm in the vaginal sperm was considered day 0 of gestation. Beginning on Day 6 and continuing daily through Day 10 of gestation, groups (19-23/group) of pregnant females were given 0, 6, 28, 130, or 600 mg/kg bw of the test material (FDA 71-28) by gavage in corn oil. A positive control group received 250 mg/kg bw/day of aspirin. Body weights were recorded on days 0, 6, 8, 10, and 14 of gestation. Females were observed daily for appearance and behavior. Food consumption and body weight were monitored to eliminate any abnormalities that may be associated with anorexia in pregnant females. On Day 14 all dams were subjected to Caesarian section and the number of implantation sites, resorption sites, live fetuses, dead fetuses, and body weight of live pups were recorded. Gestation index, mortality, gross pathology incidence of the dam urogenital tract, number of implantation sites, number of corpora lutea, litter size and weights, sex and sex ratio of pups, and gross abnormalities to pups were reported (these data were described in the robust summary for reproductive effects for the test material). The urogenital tract of each dam was examined for anatomical abnormalities. One-third of fetuses of each litter underwent detailed visceral examination at 10x magnification. The remaining two-thirds were stained with alizarin red S dye/KOH and examined for skeletal defects (the maternal and developmental fetal effects are discussed in this robust summary). |
| **NOAEL(NOEL) maternal toxicity** | >600 mg/kg bw/day (approximately equal to a daily dose of 120 mg/kg bw for allylalkoxybenzene derivatives) |
| **NOAEL (NOEL) developmental toxicity** | >600 mg/kg bw/day |
| **Actual dose received by dose level and sex** | 0, 6, 28, 130, or 600 mg/kg bw of the test material (FDA 71-28) |
| **Maternal data with dose level** | Daily clinical observation and measurement of body weight gain failed to show any differences between control and test groups of female rats. The number pregnant and % pregnancy were similar for all dose and control groups. No abortions were observed in any group. |
| **Fetal Data with Dose Level** | The average fetal weight of treatment and control groups were not statistically different ($p>0.05$). The total number of live fetuses were similar for test and control groups. A small % of (less than 3%) dead fetuses were observed at the three highest dose levels. Skeletal examination of sternebrae showed no significant differences in the incidence of incomplete ossification or missing sternebrae for test and control groups. Likewise the incidences of fetuses with more than 13 ribs, incomplete ossification of vertebrae and extremities, incomplete skull closures were similar for test and control animals. Visceral examination failed to reveal any evidence of abnormalities at any dose level. |
| **Conclusion Results** | There was no evidence of maternal toxicity or developmental toxicity at dose levels up to and including 600 mg/kg bw/day of |

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| | test material. |
| **Data Qualities Reliabilities** | Reliability code 2. Reliable with restriction. |
| **Remarks for Data Reliability** | Code 2. Acceptable, well-documented publication/study report, which meets basic scientific principles. |
| **References** | Morgareidge K. (1973b) Teratologic evaluation of FDA 71-28 in hamsters. Contract No. FDA 71-260. Unpublished report. |